Periodic patterns of two cell fates in plant roots

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Abstract: In *Arabidopsis thaliana's* root growth, several stem cells differentiate to become protophloem cells. Those cells that do not differentiate become gap cells. This differentiation process is initiated when there are high amounts of a hormone called auxin within the cell. We study the transport of this hormone through the membrane of the cells as a dynamic system in order to study the conditions and mechanisms for gap cells to appear taking as reference the published article [1]. To do so, we simulate a model of one cell, a model of two cells and a model of multiple cells. From these simulations we conclude, as proposed and shown in [1], that bistability in one cell and a lateral inhibition mechanism between cells play a key role in the emergence of phenotypes with both gap cells and differentiated cells. For bistability and lateral inhibition to take place, the auxin available for the cells must not be too low nor too high.

I. INTRODUCTION

Dynamic systems have proven to be very useful when applied to biological systems since they enable us to model a particular process so we can predict its outcoming result given certain initial conditions. These predictions, besides from telling us what is going to happen in the system, they can also help us to verify the model (if we can compare the predictions to experimental data) and thus help us to get a better understanding of the process [2].

In the work presented herein we use dynamic systems to study the spatial organization of a cellular process of differentiation occurring in the root of the model organism *Arabidopsis thaliana*. When roots grow, newly divided cells become differentiated over time into distinct cell types that perform different functions each. One cell type is protophloem. Protophloem cells transport phloem sap, which contains sugar sucrose and other photosynthesis' products that are vital for the plant survival. Protophloem cells appear next to each other forming a long chain for phloem sap transport. For a cell to become protophloem, it must have high levels of a vegetal hormone named auxin [1-3]. Therefore, if the levels of auxin are high the (stem) cell becomes a protophloem cell, otherwise the cell does not differentiate and it becomes what we call a 'gap cell' in the protophloem file of cells [1].

The dynamic system we study herein describes the transport of this hormone auxin through the cells' plasma membrane. As the hormone is both present inside (interior auxin, a) and outside (exterior auxin, a_e) cells, this transport can either bring exterior auxin inside the cell (influx) or take auxin outside the cell (efflux). The study of the auxin transport enables us to eventually determine the level of auxin inside cells in the equilibrium state and thus, know if the cell is able to differentiate or not.

When auxin goes from outside the cell into the cell, it becomes charged because of the distinct pH and is trapped inside (it cannot go outside the cell by diffusion). Therefore, auxin needs an active transport, which means a molecule (the carrier) binds with the auxin and transport it through the membrane [3-4]. Meanwhile, auxin influx is either possible by active transport or diffusion [3].

We will be taking into account two different membrane proteins that carry out the active transport of auxin, and whose effects have been seen to be relevant (see, for instance, [3-4] for modeling):

1. *PIN (P):* PIN-FORMED are auxin efflux carriers localized at the cell's plasma membrane that enable the transport of auxin from inside the cell to outside (to the extracellular medium between cells).

2. *AUX1 (I):* It is a carrier protein that facilitates the influx of auxin from the extracellular medium to inside the cell.

The efflux and influx rates increase with the concentration of PIN and AUX1 carriers, respectively. It has also been proven that the rise of the auxin levels inside the cell stimulates an increase of AUX1 production in the cell [1]. Therefore, it exists a positive feedback: the auxin level raises when transported inside the cell, which induces the cell to produce more AUX1, that at its turn will increase the amount of auxin carried inside the cell. Due to the positive feedback, a high level of AUX1 also means the cell will differentiate, as it indicates a high level of auxin inside the cell. And a low level of AUX1 also indicates low level of auxin inside the cell, so the cell becomes a gap cell.

The study presented here is centred in three models. In the first one we study a single cell given a certain amount of exterior auxin. In the second one we study two cells and we change the total amount of auxin in the system. This model enables us to study the interaction between the two cells and how they affect each other in the differentiation process. At last, we simulate a model of multiple cells in order to study the spatial organization of the differentiation process. In this last model we also change the total amount of auxin in the system.

This work is based on the scientific article entitled 'Local auxin competition explains fragmented differentiation patterns' by B. Moret and colleagues [1], which first prove that the presence of gap cells (and therefore the patterns of differentiation) is not random so there should be an underlying interaction between the cells causing them. The authors propose a lateral inhibition mechanism. This kind of mechanism lays on the assumption that a high level of auxin increases the rate of incoming auxin (by increasing AUX1). Thus, the rise of auxin levels in one cell decreases the amount of auxin level outside it and hence reduces the auxin that is available for

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the neighbouring cell. If the amount of auxin is not sufficient for all the cells to differentiate, the accumulation of auxin in some cells is going to make not possible that other cells differentiate, as there will not be enough exterior auxin available. Taking this mechanism into account the authors create a model for a single cell that they later expand to a multiple cells system.

We use the single cell model proposed in this article, slightly modified (the modifications are going to be mentioned in the following sections), and we expand it first to two and then to multiple cells, in order to study how gap and protophloem cells organize.

II. SINGLE CELL MODEL

To start with, we study a system with only one cell. In the dynamical systems approach, the cell type (or phenotype) is characterized by few states' dynamical variables. Herein, a cell is characterized by two variables: the concentration of auxin (a) inside it and the concentration of AUX1 carriers (I). The stationary state of the dynamics indicates the cell types. The possible cell types are either a differentiated cell (if in the stationary state the AUX1 levels are high) or a gap cell (if in the stationary state the AUX1 levels are low and the cell is not able to differentiate). We want to explore under which conditions each cell type appears.

For that purpose, we used a modified version of the paper's [1] equations, in which the variables have been rescaled in order to minimize the number of parameters. That is why we will be using dimensionless units. We have also added an influx of auxin due to diffusion that was not in the paper's [1] model to account for passive influx transport.

$$\frac{da}{dt} = (r_I \cdot I + r_0) \cdot a_e - r_p \cdot P \cdot a \qquad (1)$$

$$\frac{dI}{dt} = \frac{a^2}{a^2 + k_a^2} - I \qquad (2)$$

The first equation stands for the auxin (a) dynamics inside the cell and the second equation for AUX1 (I) dynamics. The value of the exterior auxin (a_e) and the PIN (P) are set constant.

For the auxin's dynamics we are taking into account two influxes: one due to the AUX1 presence (with a rate $r_I = 5 \cdot 10^4$) and another because of a diffusion process (with rate $r_0 = 100$) . The auxin is lost through the membrane due to the PIN-mediated auxin efflux (with a rate $r_p = 10^4$) .

On the other hand, the AUX1's dynamics defines the rates in which this protein is created and destroyed in the cell. The production accelerates when the level of auxin is high enough, creating a positive feedback loop. While for protein destruction the rate is proportional to the levels of AUX1 inside the cell. The equation also sets a saturation value of 1 for AUX1.

To get a better insight of the dynamics, we plot the model vector field and the nullclines (of both variables) for different values of the exterior auxin a_e.

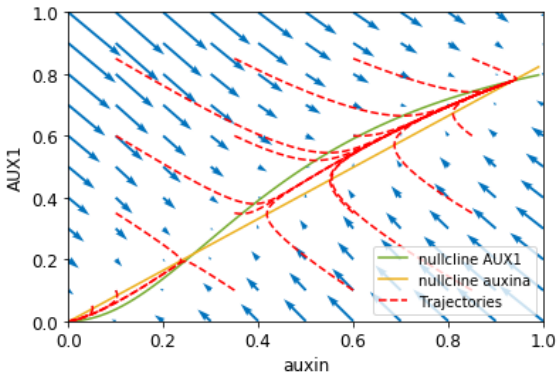


FIG. 1: Phase portrait of the single cell model. Parameters values: a_e =0.24, P = 1. The arrows are the vector field (i.e., the time derivative of each variable) and get smaller as the dynamics get slower. Red dotted lines are the trajectories from different initial conditions. These help us see the dynamics in the zones where the arrows of the vector field get too small to be seen. We also plot the nullclines for both variables.

Auxin and AUX1 nullclines are obtained by setting to zero de time derivative of a, I, respectively. Therefore, when the nullclines cross both variables are constant and we are in a stationary state [5]. Whether the state is a stable or an unstable state can be inferred from the vector field's arrows directions [5]. In figure 1 the trajectories of the variables were also plot because the vector field was hard to interpret when the arrows got too small (i.e., very slow dynamics). The dynamics of the AUX1 are slower than the auxin dynamics by a factor 10^4. This is why in the figure 1 the equation (2) has been multiplied by 10^4, otherwise it would not be possible to observe the correlation between the two variables. That is also why it is a reasonable approximation to make auxin adiabatic (quasi-steady state approximation).

From figure 1, we can see two out of the three stationary states, are stable. Hence, for those values of exterior auxin and PIN the system is bistable (we say the system is bistable when two stable states exist, and thus the final state of the system depends on the initial conditions). This means that the cell can either become a gap cell or a differentiated protophloem cell, since one of the stable state have low levels of auxin and AUX1 and the other high ones.

In order to get a deeper knowledge of the range of values of exterior auxin range where bistability is possible, we now plot a bifurcation diagram for AUX1 (Figure 2). The bifurcation diagram shows the stationary values of AUX1 for a range of values of exterior auxin.

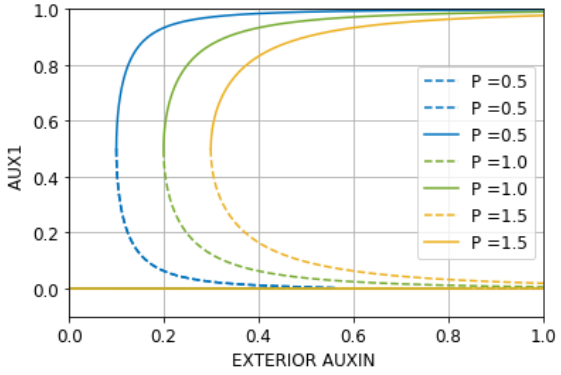


FIG. 2: Bifurcation diagrams of AUX1 for three different PIN values {0,5; 1; 1.5} each colour represents a different value of

PIN. The stable states are plot using a solid line while the unstable states are plot using a dotted line. Other parameter values as in Figure 1. The level of auxin inside the cell is higher for higher AUX1 or external auxin.

From the bifurcation diagram we gain some valuable information. First of all, it is possible to differentiate three different regions:

(1) *At low exterior auxin*: there is only one possible stationary state for the cell, which corresponds to very low AUX1.The cell becomes gap cell.

(2) *At medium exterior auxin*: there are three different stationary states. Both the states with lowest and with highest AUX1 levels are stable. Therefore, in this region the system is bistable, and the cell can become either gap or a differentiated cell.

(3) *At high exterior auxin:* there are three stationary states, but the unstable state is so close to the stable state with AUX1 ≈ 0 that any perturbation will cause the system to transition to the highest AUX1 level state. Thus, the system (with perturbations) behaves as there was just one stable state with high AUX1 and the cell differentiates.

These three different ranges are caused by the AUX1 dynamics equation, since k_a establishes a minimum value of auxin for the positive feedback to take place. Therefore, when the minimum value is not reached, AUX1 and auxin levels in the stationary state are low, thus the cell becomes a gap cell.

The other result we can extract from figure 2 is that as PIN levels increase, the bistable region appears for higher values of exterior auxin and is more extent.

III. TWO CELL MODEL

Now we extent the system by adding another cell in order to study how the auxin transport of both cells interferes with each other. This model is not presented in [1]. The dynamics of auxin and AUX1 inside each cell are:

$$\frac{da_i}{dt} = (r_I \cdot I_i + r_0) \cdot a_e - r_p \cdot P \cdot a_i \qquad (3)$$

$$\frac{dI_i}{dt} = \frac{a_i^2}{a_i^2 + k_a^2} - I_i \qquad (4)$$

where the subindex $i = 1, 2$ indicates the cell. For the transport dynamics of exterior auxin in between the cells:

$$\frac{da_e}{dt} = -(r_I \cdot (I_1 + I_2) + 2r_0) \cdot a_e + r_p \cdot P \cdot (a_1 + a_2) \qquad (5)$$

where the total amount of auxin ($a_{tot} \equiv a_e + a_1 + a_2$) is constant as we can see from equations (3) and (5):

$$\frac{da_{tot}}{dt} \equiv \frac{da_e}{dt} + \frac{da_1}{dt} + \frac{da_2}{dt} = 0 \qquad (6)$$

which enables us to work now with the constant a_{tot}, instead of the exterior auxin that in this case will depend on time.

To get a better insight on the dynamics of this model, we plot the vector field and the nullclines of $AUX1_1$ (first cell) and $AUX1_2$ (second cell) for different values of the total auxin.

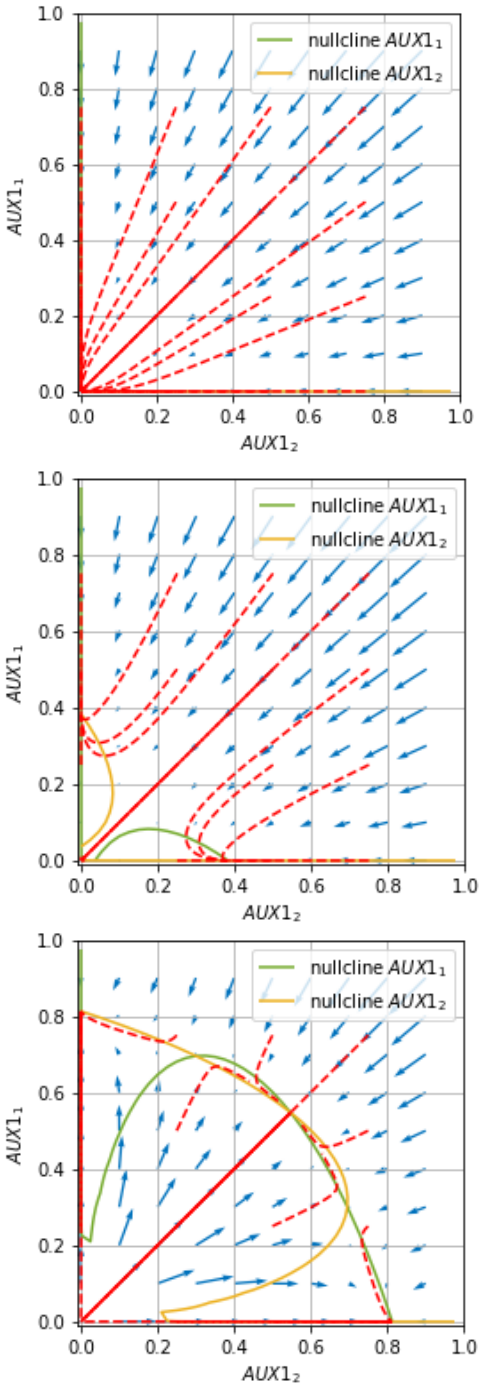


FIG. 3: Phase portraits of the linear model for two cells for two different total values of total auxin {Top, a_{tot} = 0.4; Middle, a_{tot} = 0.6; Bottom, a_{tot} = 1.3}. Parameters values: P = 1. The arrows are the vector field (i.e., the time derivative of each variable) and get smaller as the dynamics get slower. We also plot the nullclines for both variables. Red dotted lines are the trajectories from different initial conditions.

Figure 3 does not show the auxin dynamics. This is because the AUX1 dynamics is much slower than the auxin dynamics, which makes possible to assume that auxin behaves almost adiabatically. Therefore, AUX1 will control the dynamics of the system. The level of auxin is higher in the states with higher AUX1 level.

From the phase portraits three different case-scenarios can be described in terms of the total auxin:

(1) *At low total auxin* (Fig 3, Top): there is only one stationary state that is equal for both cells and corresponds to very low AUX1 and auxin ($AUX1_1 = AUX1_2 \approx 0$). Hence, there is not enough auxin for neither of the cells to differentiate, and both become gap cells.

(2) *At medium total auxin* (Figure 3, Middle): The two-gap cells stable state remains; however, two new stable states appear. In both states, one of the cells ends up with most part of the auxin available. As a result, one cell has high auxin level and differentiates and the other cell gets almost no auxin and becomes a gap cell. As the total auxin increases more two-cell trajectories end up in these states, and less in the two-gap cell state.

(3) *At high total auxin* (Figure 3, Bottom): It differs from the previous range because instead of a stable state where none of the cells differentiate, we now have a stable state where both cells have high levels of auxin and both cells will differentiate. As the total auxin amount increases, so does the range of initial conditions that its trajectory leads to this state, and less in the symmetric states.

An explanation to these results can be given if we take into account that, as mentioned before, k_a sets a minimum value of auxin for the positive feedback (that ends with high values of both AUX1 and auxin) to take place. In the low total auxin scenario, there is not enough auxin for neither of the cells to overcome the k_a minimum. While for the medium and high total auxin scenarios if the initial conditions for the cells differ enough from one another, one cell will overcome the minimum sooner than the other. The cell with the positive feedback 'activated' will increase its interior auxin, thus the exterior auxin decreases and become insufficient for the other to overcome the minimum. We are in a case of lateral inhibition. On the other hand, if the initial conditions are similar enough both cells behave the same way. If the exterior auxin levels are not sufficient for them to differentiate, they become gap cells, while if there is sufficient exterior auxin, they both become differentiated cells.

It is also relevant to take into account the power a_i(and thus, the power of k_a). So if, instead of uation (4), we now take either of the following pressions for the dynamics of AUX1 (I_i): $\frac{dI_i}{dt} = \frac{a_i}{+k_a} - I_i$; $\frac{dI_i}{dt} = a_i - I_i$ (7)

It is possible to prove that we no longer can have different values of AUX1 and auxin for each cell. Consequently, it is not possible that one cell differentiates and the other becomes a gap cell. This is because for those models bistability for one cell is not possible: there is just one stable state available for both cells, so they cannot become different types of cells.

Therefore, neither a model without the threshold that k_a sets or a power 1 for auxin (a_i), cannot describe the phenotypes observed in [1].

IV. ROW CELL MODEL

Lastly, we extent the system to a chain of N cells in order to study the spatial distribution of gap and differentiated cells for a constant amount of total auxin. The multicellular model in [1] is more complex than the one we use; [1] presents a model with a flux of auxin and polarized PINs. The model we consider is the following.

Transport of auxin inside both cells:

$$\frac{da_i}{dt} = (r_I \cdot I_i + r_0) \cdot \left(a_{e_i} + a_{e_{i-1}}\right) - 2 \cdot r_p \cdot P \cdot a_i \quad (8)$$

Where the subindex $i = 1, \dots, N$, indicates the cell. For the transport of exterior auxin in between the cells:

$$\frac{da_e}{dt} = -(r_I \cdot (I_i + I_{i+1}) + 2r_0) \cdot a_e + r_p \cdot P \cdot (a_i + a_{i+1}) \quad (9)$$

Where the subindex $i = 1, \dots, N-1$ indicates the contour conditions:

$$a_{e_0} = 0;\ \frac{da_{e_0}}{dt} = 0;\ a_{e_N} = 0;\ \frac{da_{e_N}}{dt} = 0 \quad (10)$$

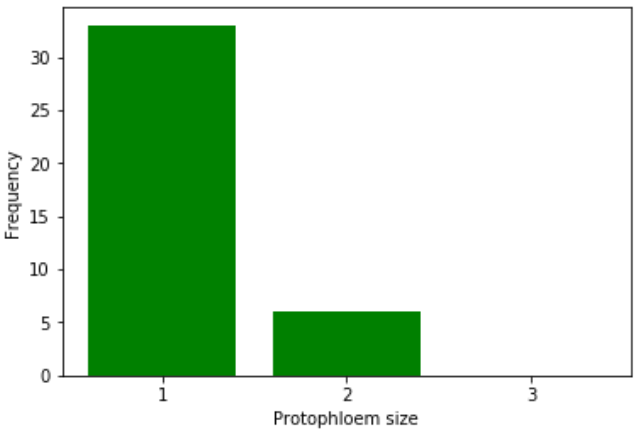
For the AUX1 dynamics we use equation (4) but now the subindex $i = 1, \dots, N$.

It can be proven that in this model the amount of auxin (a_{tot}) is also constant.

In order to determine the spatial organization of the cells we integrate the dynamic equations for a given initial conditions. For the initial conditions we set a common value of AUX1, auxin and exterior auxin for all the cells and then for each cell we add a random uniform perturbation (in a range of values) to AUX1.

For low values of total auxin, none of the cells will differentiate as there is not enough auxin available.

For medium values of total auxin, we observe that both gap cells and differentiated cells appear. Is this region we are interested in, so we proceed to analyse 19 different patterns of eight cells (N = 8) obtained in those conditions:



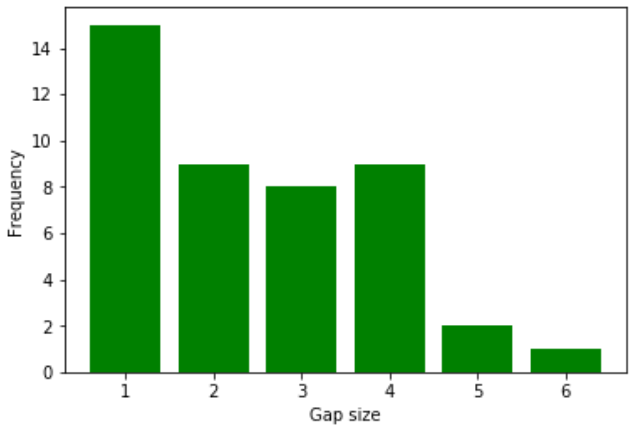


FIG. 5: Bar diagrams. The top diagram shows the frequency of the different protophloem sizes that appear (protophloem size: number of followed cells differentiated in one system). The bottom diagram shows the frequency of the different gap size that appear (gap size: number of followed cells not differentiated in one system). Those frequencies correspond to a sample of 19 equivalent simulations of the row model of 8 cells for a = 0.2, a_e = 0.2, AUX1 = 0.4, P = 1. The perturbation in AUX1 is: $\{-0.2 < \delta < 0.2\}$.

From figure 5 top we can see that major part of the cells that become protophloem cells, its neighbours become gap cells. Moreover, we have at most 2 differentiated cells next to each other. While from figure 5 bottom we observe that a greater number of gap cells their neighbours are also gap cells.

As we increase the amount of total auxin available, the frequency of higher protophloem sizes increase and higher gap sizes decrease (data not shown).

For high values of total auxin, all the cells will differentiate, as the amount of total auxin is sufficient for all the cells to overcome the minimum auxin set by k_a and as previous seen, differentiate.

V. METHODS

The simulations have been developed using python and the integrations have been computed using Runge-Kutta 4.

VI. CONCLUSIONS

Through our work we have studied the transport of auxin, regulated by the auxin's carriers, for three different systems (one, two and multiple cells). The simulations of those models have led us to conclude that for different amounts of auxin available in the system we obtain different phenotypes, for the three models. The phenotypes for two and multiple cells depend on whether bistability is possible or not for the one cell model. In the model we are studying, if bistability appears for one cell is determined by the amount of auxin available. We have two different cases: for low auxin levels we do not observe cell differentiation (all the cells become gap cells) in any of the three models, while for high auxin levels the cells will always differentiate and become protophloem in any of the three models. This last phenotype, where all the cells differentiate, corresponds to the phenotype observed in wild type roots [1].

Bistability appears for medium values of exterior auxin for the one cell model, which corresponds to medium values of total auxin for the two and multiple cells model. In this case, cells can either become a protophloem cell or a gap cell, depending on the initial conditions of interior auxin and AUX1. For those values lateral inhibition mechanism is possible the models show that both gap cells and protophloem cells appear, as seen in roots where the amount of PIN is decreased [1]. From these results it can be suggested that in these roots there is less amount of auxin available than for wild type roots [1]. Here we used an isotropic model with no flux of exterior auxin to study the periodicity of the pattern and we did not intend to mimic the phenotypes. In the two cell model, not present in the paper, we also see that lateral inhibition is possible due to AUX1 dynamics expression. We do not see a pattern for the multiple cell model, but we can infare the probability of the gap size and the protophloem for a certain amount of total auxin.

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