Mathematical biology: from individual cell behavior to biological growth and form

Final Project Report

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A pH-mediated proposed mechanism for cell signaling and auxin transport

1. Introduction

1.1 Biological background

The plant (phyto-) hormone called indole-3-acetic acid (IAA) has been found in all plants studied to date [1]. IAA is often referred to as auxin, since it is the most common member of a class of molecules with related structures known as auxins [2]. The structures of IAA and several other commonly studied auxins are depicted in Figure 1.

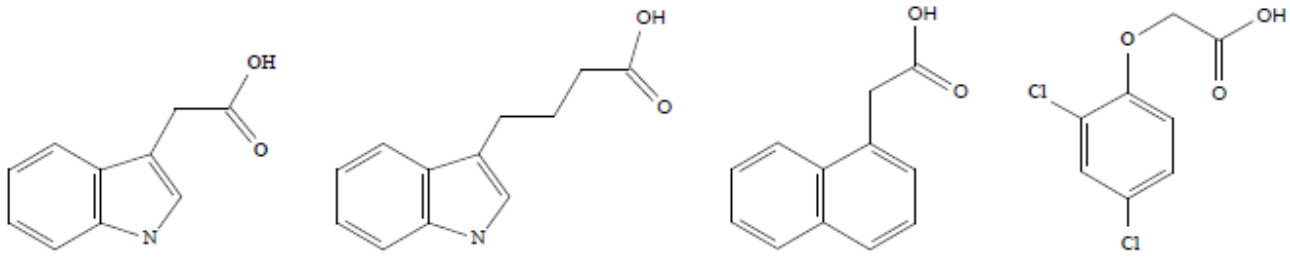

Figure 1: Structures of common auxins. Left to right: natural auxins, indole-3-acetic acid (IAA) and indole -3-butyric acid (IBA); synthetic auxins, 1-napthylacetic acid (1-NNA) and 2,4-dichlorophenoxyacetic acid (2,4-D).

In arabidopsis and other plants, auxin has an impressive range of effects. Since the identification of the hormone by Dutch scientist Frits Went in 1926 [3], it has been shown to be involved in cell expansion and division, vascular tissue specification and differentiation, root initiation, tropic responses, and various stages of fruit and flower development – in short, in all plant tissues at all stages of development [4].

Auxin is unique among known plant hormones in that it is actively moved between cells in specified directions by transporter proteins [5]. Auxin is a weak acid, with a pK_a of about 4.8 [6]. In the intercellular space (pH ≈5.5), about 15% of auxin molecules are protonated and electrically neutral, and can therefore diffuse through the cell membrane into the cytoplasm. The low pH in the apoplast (cell wall) is maintained through the activity of plasma membrane H^+-ATPases. In the more basic cell interior (pH ≈ 7), the acidic proton is lost to yield anionic IAA^-, which is unable to diffuse out of the cell.

This observation forms the basis of the long-standing 'chemiosmotic hypothesis' for auxin transport, which also postulates that the asymmetric distribution of auxin efflux transporters is largely responsible for the directionality of auxin transport (Fig. 2) [7]. The chemiosmotic hypothesis [8] was first applied to explain auxin transport in the mid-1970s, and in large part recent work has merely supplied details about the molecular players involved in the remarkable original hypothesis [9]. One important addition

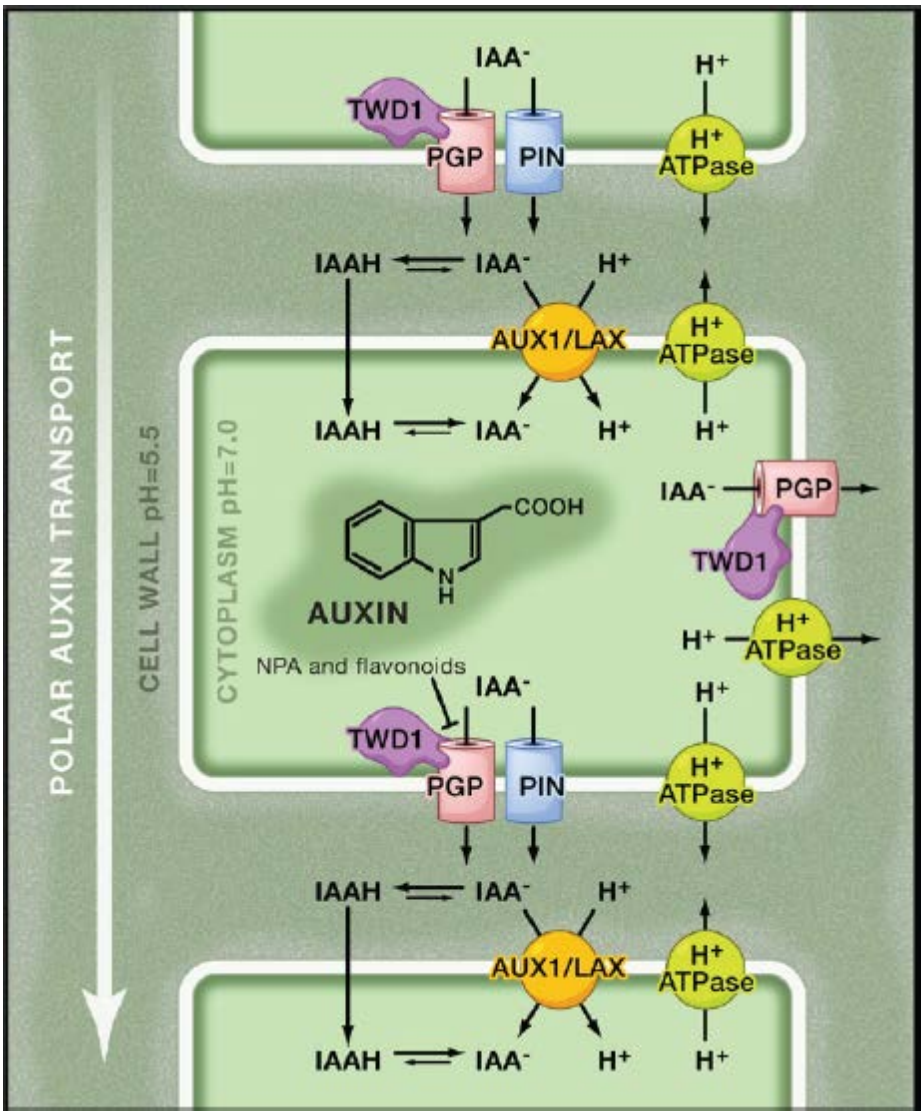

Figure 2: Schematic representation of the chemiosmotic hypothesis for auxin transport. Anionic auxin (IAA^-) is transported by specific carrier proteins (solid arrows). PIN and PGP are the efflux carriers, while AUX1 is an influx transporter. Protonated auxin (IAAH) is also able to diffuse from the extracellular space into the cell (dotted arrows), where it ionizes due to the higher internal pH.

has been the realization of the importance of active auxin transporters, not diffusion alone, for auxin influx [6]. Auxin is actively taken up from the apoplast by H^+/IAA^- symport mediated by AUX1/LAX influx carriers. Auxin can leave the cell by auxin efflux carriers such as PIN-FORMED (PIN) proteins and P-glycoproteins (PGP) of the ATP-Binding Cassette family B (ABCB) transporter family.

Eight PIN genes were discovered in arabidopsis that can be divided into two broad subfamilies according to the presence or absence of a distinct central hydrophilic loop separating two hydrophobic domains. The subfamily containing the loop ('long' PINs) encompasses all members of the family that are defined as auxin-efflux carriers localized at the plasma membranes (PIN1-PIN4 and PIN7). In addition, PIN6 is also included as a member of the long PIN subfamily on the basis of the high sequence similarity in the trans-membrane regions and only partial reduction of the hydrophilic loop. The second major PIN gene subfamily encodes proteins with the central hydrophilic loop virtually absent ('short' PINs) and

comprises *At*PIN5 and *At*PIN8. The short PINs appear to localize to a large extent to the endoplasmic reticulum, and although they presumably act as auxin transporters, they do not directly facilitate auxin transport between cells but mediate intracellular auxin compartmentalization and homeostasis.

ABCB activity can be modulated by 1-naphthylphthalamic acid (NPA) and flavonoids that interfere with the interaction of ABCB and a protein that regulates it, TWISTED DWARF 1 (TWD1). The polar subcellular localization of PINs determines the direction of auxin flow out of the cell and thus the unidirectional auxin flow within tissues.

The study of auxin and its polar transport has revealed an intricately regulated system. Complex regulation is to be expected from a system that needs to be very flexible but must always achieve a functionally similar final state to be useful. Unfortunately, it is difficult to determine auxin location directly [10], and so its presence must usually be inferred from its downstream effects.

The observation of zones of auxin flow narrowing into single auxin-transporting files of cells is reminiscent of a classic series of experiments by Sachs, in which he showed that auxin flow is capable of inducing the formation of veins (in pea stems) [11]. On the basis of these experiments, Sachs proposed the 'canalization hypothesis' [12], which posits an autocatalytic feedback between auxin flow and auxin transport capacity. Cells with high levels of auxin flow undergo changes that make them more efficient at transporting auxin. When coupled with an asymmetry of efflux transporters, as in the chemiosmotic hypothesis, auxin produced in one zone becomes 'canalized' into files of cells specialized for auxin transport (Fig. 3).

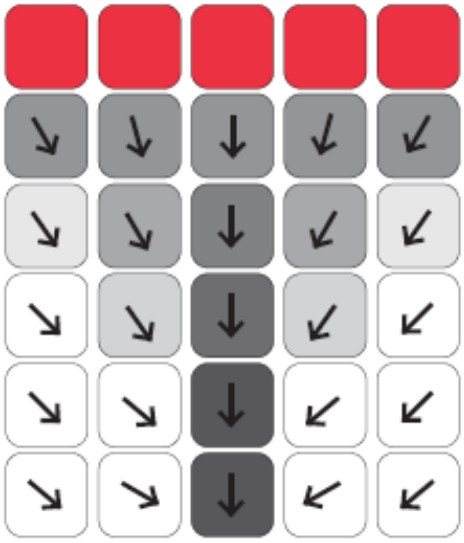

Figure 3: Schematic representation of the canalization hypothesis. Auxin produced in leaf margin cells (top row) is transported basally. Random variations in transport result in one cell having a slightly higher auxin flux than its neighbors. That cell thereby becomes better at transporting auxin, which induces higher flux in the cell directly below it, and so on. High-flux cells, since they rapidly export their auxin contents, become sinks for the auxin of neighboring cells, and positive feedback results in a file of cells (a 'canal') with high auxin transport capacities. Cell shading indicates relative auxin flux intensity.

A current challenge of the proposed biological models is the existence of a signal that conveys to a cell the information about the auxin concentration of neighboring cells. Also, it remains unknown how this signal is conveyed to the mechanism that controls PIN protein trafficking. The goal of this proposal is to

address the first question that is which signal is responsible for a cell tracking the auxin content of its neighbors.
The various computer models stemming from the different biological hypotheses will be discussed in the next section.

1.2 Computer Models

Considering the existing computer models for polar auxin transport, an appropriate starting point would be to think of the process as a trans-cellular process, where auxin moves from the cytoplasm of one cell to the next by crossing both cell membranes and the cell wall that separates them. When trying to model such a process the most logical question would be how to describe a cell to make a computer simulation possible. This is called discretization and has to do with the level of abstraction used to describe a cell. A number of boxes is used to represent one or more cells and according to this number a different resolution is obtained.
There are high resolution models, where cells are modeled with enough detail (enough boxes) to represent the concentrations in subcellular compartments and the apoplast. Thus, an accurate subcellular auxin gradient is depicted as is the U-shaped distribution of efflux carriers on the cell membrane [13, 14].
Then, there are medium resolution models, which are the most popular, that assign exactly one box to one plant cell [15 – 17]. These models cannot capture the subcellular auxin gradient, nor can they resolve the distribution of auxin within different subcellular compartments (vacuole, cytoplasm, nucleus, etc.). Of course, these models carry the implicit assumption that such details are not relevant to the investigated topic and that assumption will later be discussed.
Finally, there are low resolution models, where tens to thousands of cells are assigned to one box [18, 19]. The main advantage of such an approach is economy, as whole plant organs can be simulated in relatively short computer time.
As the hypotheses for the biological aspects of the phenomenon evolved, so did the simulation models. For example, the canalization hypothesis provided the impetus for a series of modeling papers by Mitchison in the early 1980s [15, 16]. Mitchison's models involve feedback between auxin flux and some parameter of the transport process. He first [15] considered purely diffusive auxin transport in an array of cells, with a linear auxin source and sink at the top and bottom of the array, respectively. Mitchison's diffusion-based model was able to reiterate some phenomena observed by Sachs [11], such as the 'repellent' effect of nearby strands if both are carrying high auxin fluxes, and the formation of cross connections from new sources to previously formed strands with low flux.
Diffusion alone, however, could not account for some observed phenomena, such as vein loops with circulatory flow. Mitchison therefore also considered models with polar auxin transport, particularly in a subsequent paper [16]. This model supposes auxin pumps or channels at one end of each cell, whose number or transport efficiency increases with increasing auxin flux.
Although, this is an indicative example of evolving computer models as the biological assumptions change, there are still some unanswered questions. To that effect, this proposal hypothesizes that a computer model may give additional support for the biological process involved in cell signaling.

2. Proposed Model

2.1 Hypothesis

Throughout the literature there have been indications that PIN proteins are gradient-driven secondary transporters and that a H^+ membrane gradient in particular is responsible for auxin efflux [20, 21]. This fact is not correlated throughout the literature and it seems that there is some confusion regarding the matter. The question of how cells are aware of their neighbors' auxin content still remains.
In 1898, Henry Louis Le Chatelier delivered a principle that would be named after him [22]. Le Chatelier's principle postulates that: "*If a chemical system at equilibrium experiences a change in concentration, temperature, volume or pressure, then the equilibrium shifts to counteract the imposed change and a new equilibrium is established*".
This principle is the starting point of the current hypothesis. Auxin is a weak acid with a pK_a of about 4.8. This means that both in the cytoplasm and the apoplast the following equilibrium exists:

$$IAAH \rightleftarrows IAA^- + H^+, \qquad (1)$$

shifted accordingly due to the difference in pH (7.0 and 5.5 respectively). As auxin defuses into and is actively taken up by the cells the change in concentration of the ionized and protonated forms of auxin will influence the equilibrium according to Le Chatelier's principle. In turn, the change of the equilibrium will change the proton content (pH) of the cells and protons will be pumped in or out by the H^+-ATPase membrane pumps. A detailed description of this process will be given below.

2.2 Model Description and Assumptions

Let Cell 1 be a square plant cell with a high auxin content (Fig. 4, {1}) and an adjacent square cell, Cell 2, have a lower auxin content relative to the first one (Fig. 4, {2}). Auxin will be mostly in the ionized form (IAA^-) due to the neutral pH inside the cell. The excess of protons in Cell 1 will be pumped out of the cell by H^+-ATPase pumps (Fig. 4, {3}) and into the cell wall. Due to the loss of protons, equilibrium (1) shifts to the right, so that more H^+ can be generated. This results in less IAAH inside Cell 1 (Fig. 4, {4}). As there is a change of proton concentration in the cell wall, equilibrium (1) shifts to the left (Fig. 4, {5}) yielding more IAAH. Thus, a sufficient IAAH gradient is generated to stimulate diffusion of IAAH from the cell wall to Cell 1 (Fig. 4, {6}).
Meanwhile, Cell 2 has four "options" to pump auxin out from its four sides. The side of the apoplast that is in touch with Cell 1 has become more acidic (Fig. 4, {7}), so there is a steeper proton gradient. Since PINs and AUX/LAX carriers are energized by the proton motive force, a change in the proton gradient over the membrane might also lead to changes in carrier-mediated transport. This would result in Cell 2 having a higher probability to pump its auxin content to the side in touch with Cell 1 (Fig. 4, {8}).
Thus, a positive feedback loop of auxin and protons is hypothesized with the underlying mechanism that a higher cellular auxin concentration leads to a more acidic extracellular space, which in turn causes more protonation of extracellular auxin and hence more auxin to enter the cell, and so on. Moreover,

changes in the proton gradient might be the indirect signal for a cell to know which of its neighbors has the higher auxin content.

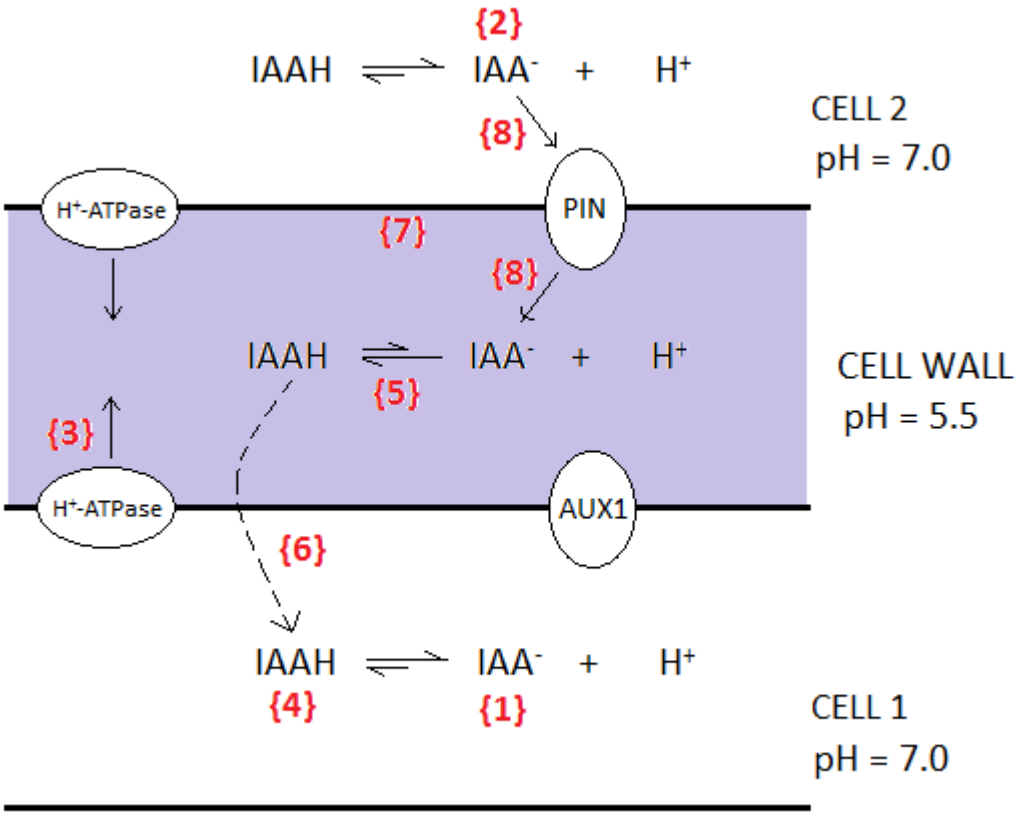

Figure 4: Schematic representation of the proposed hypothesis. Numbers {1} through {8} explained in text above.

The first assumption that is made is that the cell is a non-homogenous space (to allow for diffusion) with no subcellular compartments. For example, there is no vacuole or endoplasmic reticulum. This simplification reduces the complexity of the proposed model and focuses only on the changes happening due to trafficking of protons and auxin in and out of the cell.

The main assumption made for this model is that the proton concentration changes caused by Le Chatelier's principle will actually be measurable and comparable to the effects of IAA^- transport. If they are not comparable, then – for all intents and purposes – it would mean that the proton change cannot be accountable for the shifts of the equilibrium in and out of the cells and the chemical cascade that follows. Due to the fact that the volumes and concentrations are infinitesimal, it is believed that proton fluctuations indeed play an important role in the overall process.

Another equally important assumption is that no buffer is used for the compartmental pH. The pH values in the cytoplasm and apoplast are considered constant at 7.0 and 5.5 respectively plus the changes inferred from a single species of one mono-protic acid per compartment (in this case auxin).

This is a simplification as pH buffering is a complex process that relies on a mixture of *n*-protic acids, but in this model the focus was only on the changes made by protons generated from auxin dissociation.
A fourth assumption is that H^+-ATPase pumps do not strictly rely on ATP to be functional.
In literature some supporting evidence could be found for the current hypothesis, especially the positive feedback loop between protons and auxin [23 – 25]. It was encouraging to realize that this pH-mediated mechanism hypothesis was supported by others and was tested in other papers, especially [26].
In this proposal, two sets of differential equations are used. One set is to track concentrations inside the cell and the other outside the cell, in the cell wall.

2.3 Equations

Some terms are the same in all equations.

Set 1 – Inside the cell

Equation (1):

$$\frac{\mathrm{d}[\mathrm{IAAH}]}{dt} = K_1 + K_2[IAA^-](10^{-7} + [H^+]) + D\nabla[IAAH] - K_3[IAAH] - K_4[IAAH]$$

where, [IAAH], [IAA^-] and [H^+] are the concentrations of protonated and deprotonated forms of auxin and protons. K_1 is a constant representing the rate of production of auxin by the biological processes of the cell. K_2 represents the rate of protonation of IAA^- (left direction of equilibrium (1)), D is the diffusion coefficient, K_3 is the rate of IAAH de-protonation (right direction of equilibrium (1)) and K_4 is the degradation rate of auxin.

Equation (2):

$$\frac{d[IAA^-]}{dt} = -K_2[IAA^-](10^{-7} + [H^+]) + K_3[IAAH] - K_5[IAA^-]$$
$$-\sum_{i=1}^{n} C_{PIN}(10^{-5.5} + [H^+]_{Wall_i})PIN_{Wall_i} + \sum_{i=1}^{n} C_{AUX1}(10^{-5.5} + [H^+]_{Wall_i})[IAA^-]_{Wall_i}$$

where, K_5 is the degradation rate of IAA^-. C_{PIN} is a constant representing the amount of IAA^- that one PIN protein can pump out of the cell. According to the neighborhood that is assigned to every cell there may be any number from 2 to 8 adjacent cell walls, $Wall_i$. PIN_{Wall_i} is the number of PINs that pump auxin to $Wall_i$. These two are multiplied with the proton content in each of the walls, to demonstrate that PINs are energized proportionally to the cell wall proton content. The sum represents the amount of IAA^- that is flushed out of the cell. The second sum represents the amount of IAA^- that gets in the cell due to active transport by the AUX1 carriers. C_{AUX1} is a constant representing the amount of IAA^- that a single AUX1 protein pumps in the cell. $[IAA^-]_{Wall_i}$ is the auxin concentration in each $Wall_i$. Again to

demonstrate the proportionality of active carriers to the proton and auxin content of each adjacent wall, C_{AUX1} is multiplied by the wall proton content.

Equation (3):

$$\frac{d[H^+]}{dt} = -K_2[IAA^-](10^{-7} + [H^+]) + K_3[IAAH] - K_6(10^{-7} + [H^+]) - C_{H-Pump}(10^{-7} + [H^+])$$

where, K_6 is the buffering rate of protons in the cell. The last term represents the amount of protons pumped out of the cell due to H^+-ATPase pumps. $C_{H\text{-}Pump}$ is a constant representing the capacity of a single H^+-ATPase pump to pump protons out of the cell.

Set 2 – Inside the Cell Wall

The logic is similar to the previous set of equations, with one characteristic difference; no matter what cell neighborhood is implemented, the cell wall only comes in contact with two cell membranes.

Equation (4):

$$\frac{\mathrm{d}[\mathrm{IAAH}]}{dt} = K_2[IAA^-](10^{-5.5} + [H^+]) + D\nabla[IAAH] - K_3[IAAH]$$

The absence of K_1 signifies that auxin is only produced inside the cell. The degradation term is missing as well, as it is assumed that degradation of IAAH does not take place in the cell wall.

Equation (5):

$$\frac{d[IAA^-]}{dt} = -K_2[IAA^-](10^{-5.5} + [H^+]) + K_3[IAAH] + \sum_{i=1}^{2} C_{PIN}(10^{-5.5} + [H^+])PIN_{Cell_i} - 2C_{AUX1}[IAA^-](10^{-5.5} + [H^+])$$

The degradation term is not present in equation (5) as it is assumed that no degradation takes place in the cell wall. The contribution of PINs to the concentration of IAA^- is limited to the two cells adjacent to every cell wall. The last term accounts for the amount of auxin leaving the cell wall due to active transport. The term $2C_{AUX1}$ signifies that the amount that AUX1 carriers can flux into the cells is doubled, considering the viewpoint of the cell wall.

Equation (6):

$$\frac{d[H^+]}{dt} = -K_2[IAA^-](10^{-5.5} + [H^+]) + K_3[IAAH] - K_7(10^{-5.5} + [H^+]) + \sum_{i=1}^{2} C_{H-Pump}\left(10^{-7} + [H^+]_{Cell_i}\right)$$

where, K_7 is the buffering rate of protons in the cell wall. The last term accounts for the contribution of protons from the two cells adjacent to the cell wall.

PIN Reorientation

In every time-step of the algorithm a certain probability is assigned for the PINs to re-orientate to a different cell wall.

Equation (7):

$$P = C\frac{[IAA^-]}{[IAA^-] + [IAA^-]_{Wall_i}}$$

If this probability is below a certain threshold, PINs stay oriented facing the same cell wall otherwise they are re-assigned to one of the remaining walls with equal probability. C is a scaling constant to better balance the probability. Equation (7) implies that the more auxin there is in a cell and its adjacent wall, the smaller the probability is for the PINs to orientate elsewhere. This probability is updated in every time-step after the calculation of IAAH, IAA^- and H^+ concentrations.

3. Results

Following the formulation of the model, an implementation was made in R [27]. The implementation consists of a row of 20 alternating cell walls (10) and cells (10). An initial random IAAH concentration is assigned randomly to every cell, as these are the only ones that produce IAAH in the model. From the very first moments of the simulation, it is apparent that the cells initialized with a higher IAAH concentration, are the ones accumulating more IAA^- and H^+ as well (Fig. 5). This is to be expected, as these cells generate more IAA^- and H^+ from IAAH dissociation, since equilibrium (1) is shifted to the right. The IAAH concentration in the cell walls is very low as equilibrium (1) shifts to the left creating a steep gradient that allows IAAH to diffuse into the cells.

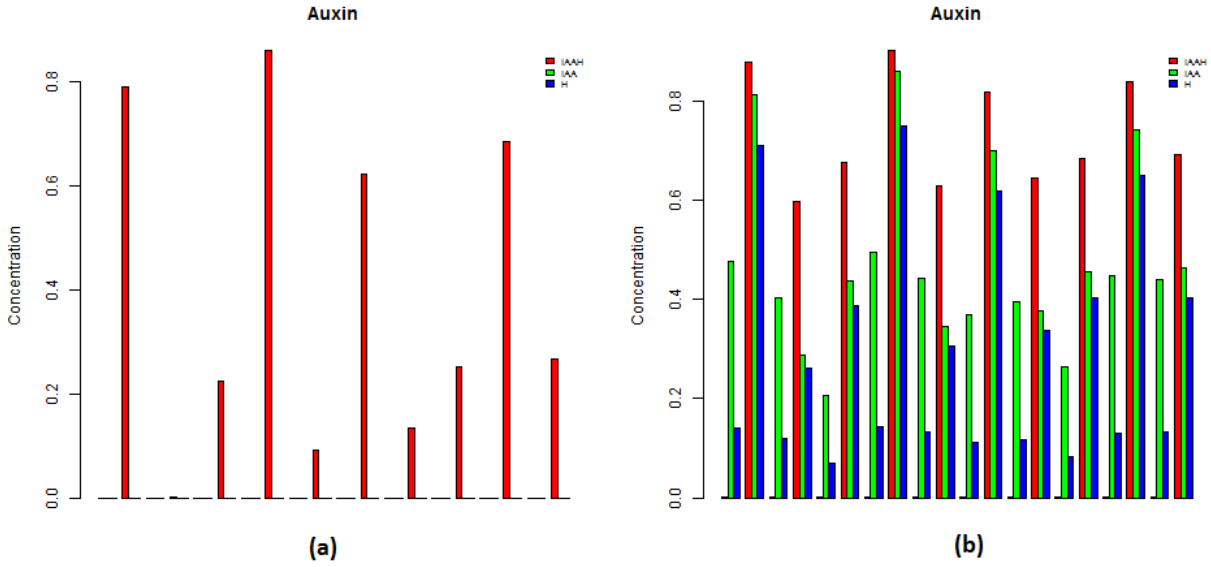

Figure 5: (a) Random initialization of cell IAAH concentration. (b) After a few iterations, the cells with high IAAH content also accumulate larger amounts of IAA^- and H^+ as well. The IAAH content of the cell walls is very low, as it diffuses to the cells. IAAH in red, IAA^- in green and H^+ in blue. The array starts with a cell wall and ends with a cell, alternating between cells and cell walls.

The assumption made that a cell wall that becomes more acidic can trigger an adjacent cell with lower auxin to pump its contents to that wall, holds in the simulation. As can be seen in Fig. 6, Cell 2' has a lower auxin content compared to Cell 1'. Cell wall 2, adjacent to Cell 1', has a higher proton content compared to Cell wall 3, adjacent to Cell 2'. As Cell wall 2 is more protonated, its IAA^- content is also higher compared to Cell wall 3, as Cell 2' pumps its contents to Cell wall 2 rather than 3.

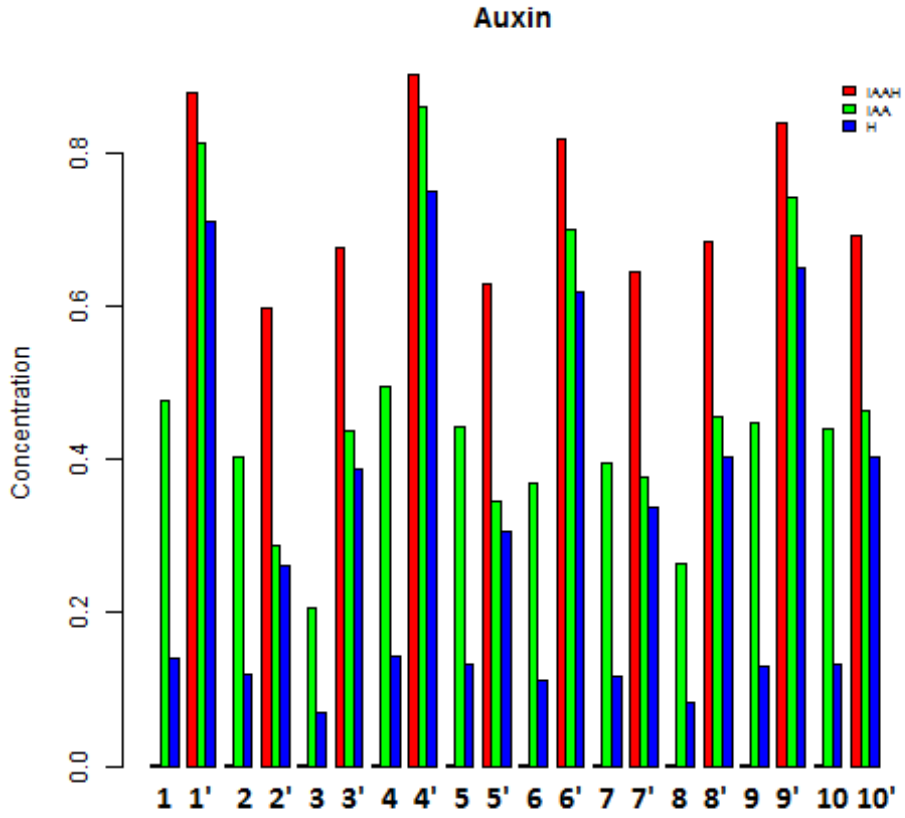

Figure 6: Higher protonation of a wall makes it the favorable path for adjacent cells to pump their auxin to. Normal numbers are cell walls, prime numbers are cells.

Unfortunately, as the simulation progresses, the cells first become saturated with deprotonated auxin. As this happens, IAAH concentration reaches a steady state that then enforces all the other parameters to fall to a steady state as well. The cell wall IAA^- concentration drops slightly and becomes similar for all the cell walls. Afterwards, all other concentrations become almost equal and no more change is observed, as can be seen in Fig. 7. This was an unpredicted behavior of the implementation. A measure to counteract it would be to have a cut-off concentration for the cells, so that they would not be able to accumulate auxin indefinitely. This solution did not work though. The problem is that the implementation is not biologically realistic, because cells have a steady production of auxin. In reality, cells would not produce auxin continuously if an internal signal was generated after a certain threshold was reached. This could be solved by making the production rate inversely proportional to another concentration in the system.

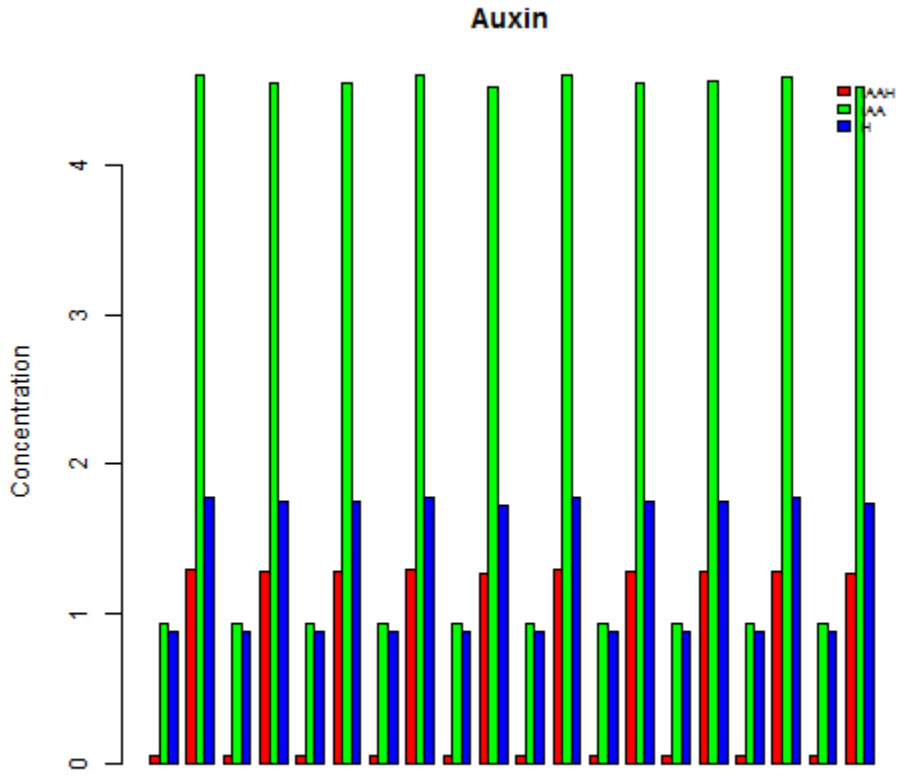

Figure 7: System in steady state. All concentrations very similar to each other between cells and cell walls.

The parameters used in the simulation can be seen in Table 1.

Parameter	Description	Value
K_1	Production rate of IAAH	0.101
K_2	Protonation rate of IAA^-	0.005
K_3	De-protonation rate of IAAH	0.1
K_4	Degradation of IAAH	0.01
K_5	Degradation of IAA^-	0.1
K_6	Buffering of protons	0.0
K_7	Buffering of protons in cell wall	0.2
C_{PIN}	IAA^- one PIN protein can pump	0.07
C_{AUX1}	IAA^- one AUX1 protein can pump	0.15
D	Diffusion rate	10^{-4}
$C_{H\text{-}pump}$	H^+ one H^+-ATPase pump can pump	0.05
pH_{cell}	pH of cell	10^{-6}
pH_{wall}	pH of cell wall	10^{-4}

Table 1: Parameter values used in the simulation.

4. Discussion

The proposed model links auxin transport to ionic transport, providing insights into the feedback mechanism dynamics between auxin and protons. According to this hypothesis, there are some interesting remarks to be taken into account. First, the model leads to increased diffusion of auxin by changing auxin dissociation status in the apoplast. Second, it builds up a steeper proton gradient over the cellular membrane, increasing the carrier mediated transport rates for auxin, which are energised by this proton motive force. Third, it leads to a pronounced allocation of auxin inside cells. Extending the work in [26], the model expands to tracking the behavior of multiple cells and the repercussions that proton trafficking has for signal transduction. In our opinion, this model can serve as a viable basis to give useful insights for unexplained assumptions of other models. For example, it could help explain the assumption of the signal polarizing auxin transport according to flux, made for the canalization hypothesis [11, 16]. This project provided great insight into multicellular processes and was considered a great opportunity to combine multiple disciplines.

References

1. Woodward AW & Bartel B (2005) Auxin: Regulation, action and interaction. *Ann Bot* **95**, 707–735.
2. Kende H & Zeevaart JAD (1997) The five "classical" plant hormones. *Plant Cell* **9**, 1197–1210.
3. Went, F. (1935). Auxin, the plant growth hormone. *Bot. Rev.* **1,** 162–182.
4. Perrot-Rechenmann C & Napier RM (2005) Auxins. In *Vitamins and Hormones*, vol. 72, pp. 203–233, Elsevier.
5. Goldsmith MHM (1977) The polar transport of auxin. *Ann Rev Plant Physiol* **28**, 439–478.
6. Kramer EM & Bennett MJ (2006) Auxin transport: A field in flux. *Trends Plant Sci* **11**, 382–386.
7. Raven JA (1975) Transport of indoleacetic acid in plant cells in relation to pH and electrical potential gradients, and its significance for polar IAA transport. *New Phytol* **74**, 163–172.
8. Mitchell P (1961) Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. *Nature* **191**, 144–148.
9. Estelle M (1998) Polar auxin transport: New support for an old model. *Plant Cell* **10**, 1775–1778.
10. Schlicht M, Strnad M, Scanlon MJ, Mancuso S, Hochholdinger F, Palme K, Volkmann D, Menzel D & Baluska F (2006) Auxin immunolocalization implicates vesicular neurotransmitter-like mode of polar auxin transport in root apices. *Plant Signal Beh* **1**, 122–133.
11. Sachs T (1969) Polarity and the induction of organized vascular tissues. *Ann Bot* **33**, 263–275.
12. Sachs T (1981) The control of the patterned differentiation of vascular tissues. *Adv Bot Res* **9**, 152–262.
13. Kramer EM (2004) PIN and AUX/LAX proteins: their role in auxin accumulation. *Trends in Plant Science* **9**, 578–582.
14. Swarup R, Kramer EM, Perry P, Knox K, Leyser HMO, Haseloff J, Beemster GTS, Bhalerao R, Bennett MJ. (2005) Root gravitropism requires lateral rooot cap and epidermal cells for transport and response to a mobile auxin signal. *Nature Cell Biology* **7**, 1057–1065.
15. Mitchison G (1980) A model for vein formation in higher plants. *Proc R Soc Lond B* **207**, 79–109.
16. Mitchison G (1981) The polar transport of auxin and vein patterns in plants. *Phil Trans R Soc Lond B* **295**, 461–471.
17. Smith RS, Guyomarc'h S, Mandel T, Reinhardt D, Kuhlemeier C, Prusinkiewicz P (2006) A plausible model of phyllotaxis. *Proc Nat Acad Sc, USA* **103**, 1301–1306.
18. Mitchison GJ (1977) Phyllotaxis and the Fibonacci series. *Science* **196**, 270–275.
19. Dimitrov P, Zucker SW. 2006. A constant production hypothesis guides leaf venation patterning. *Proc Nat Acad Sc, USA* **103**, 9363–9368.
20. Lomax TL, Muday GK, Rubery PH (1995): Auxin transport. In *Plant Hormones: Physiology, Biochemistry and Molecular Biology*, 2nd edition. Edited by Davies PJ. Dordrecht, Boston, London: Kluwer Academic Publishers; 509-530.
21. Křeček P, Skůpa P, Libus J, Naramoto S, Tejos R, Friml J, Zažímalová E.: The PIN-FORMED (PIN) protein family of auxin transporters, *Genome Biology* 2009, **10:**249
22. Le Chatelier, H. and Boudouard O. (1898), Limits of Flammability of Gaseous Mixtures, *Bulletin de la Société Chimique de France* (Paris), **19**, pp. 483–488.
23. Fitzsimons, P.J. (1989) The determination of sensitivity parameters for auxin- induced H^+-efflux from Avena coleoptile segments. *Plant Cell Environ*, **12**, 737–746.
24. Hager, A., (2003) Role of the plasma membrane H^+-ATPase in auxin-induced elongation growth historical and new aspects, *J. Plant Res.*, 116 (**6**), 483–505.
25. Leyser, O., (2005), Auxin distribution and plant pattern formation: how many angels can dance on the point of PIN? *Cell*, 121 (**6**), 819–822.

26. Steinacher A, Leyser O, Clayton RH, (2011), A computational model of auxin and pH dynamics in a single plant cell, *J Theor Biol*, **296** (2011) 84–94
27. R Development Core Team, (2006) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.