# Hormonal and Cytoskeletal Regulation of Root Growth

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# Contents

# Chapter 1

# Introduction

Root growth in *Arabidopsis Thaliana* is a tightly regulated process governed by a network of interacting hormones. Central to this network is the plant hormone auxin, which has been linked to nearly every aspect of cell behaviour within the root [27]. Additionally, at least eight other substances [31], including cytokinin, brassinosteroids (BR), and ethylene have been shown to contribute to promoting robust and adaptable hormone gradients within the root. The extensive measurement of these hormones, their derivatives, and behaviour [3, 14, 18, 32] has provided fertile ground for mathematical modelling. While various approaches have been employed in modelling these hormone gradients [26], this paper will explore two dimensional models on a vertical cross section of the static or growing root.

Researchers have done considerable work on modelling auxin-cytokinin crosstalk [4, 6, 17, 21, 23, 28] and some research into auxin-ethylene crosstalk [22]. However, mathematical models of auxin-BR crosstalk have been limited to a horizontal cross section of a static root [11]. Additionally, only a small subset of existing models incorporate root growth, which produces important feedback loops with the hormone gradient [26]. It is from these observations that the research questions for this paper are identified:

- How does BR influence auxin distributions in static and growing roots?

- Is BR-auxin crosstalk sufficient to replicate experimentally identified growth rates across the root?

This paper proceeds with a brief overview of biological information about *Arabidopsis* roots, followed by an exploration of prior modelling methods and

results. Then, a model incorporating auxin, cytokinin, ethylene, and BR will be developed and tested against previous results.

## 1.1 Root Morphology

### 1.1.1 Structure and Regions

The interactions between auxin and other hormones partitions the root into four zones with distinct growth activities [32].

Surface

Mature Zone (MZ)

Elongation Zone (EZ)

Transition Zone (TZ)

Division Zone (DZ)

Root Tip
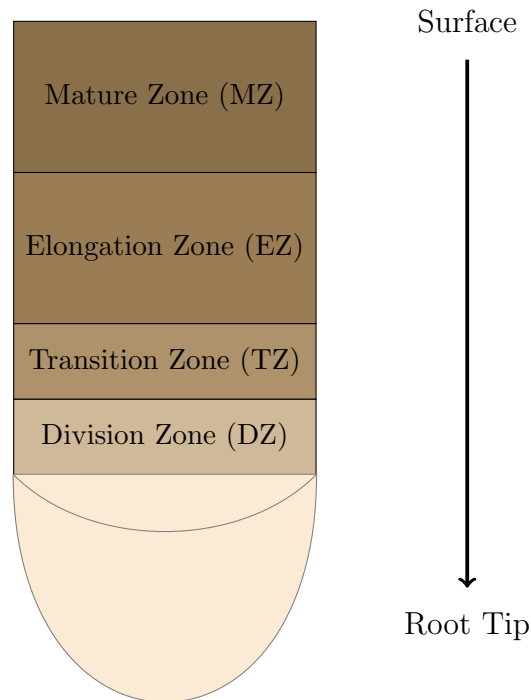
Vascular, border, and epidermal cell columns make up the primary root shaft. The flow of the various hormones between these columns will be explored further in later sections. Underneath the columns of cells is the root cap, which protects the root as it grows [16]. In order to preserve this function, root cap cells are continuously created and destroyed [16], and thus

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most models of the growing root assume the root tip is static [17, 28]. At the top of the root cap is the important quiescent centre (QC), which aids in regeneration of the root cap and promotes division of the cells around it [19].

The division zone spans up to 200 μm away from the root cap junction. In this region, cells grow from 4.5 μm up to 9 μm over the course of approximately 18 hours. On average, the division zone contains around 21 cells per file, and all cells are mitotic. The transition zone ranges from the top of the division zone to about 520 μm above the root cap junction. Cells in this zone grow to 30 μm over 10 hours. In the transition zone, vascular cells are not mitotic, but border and epidermal cells are. Rapid cell elongation occurs in the next region, up to 900 μm above the root cap junction. Cells in the elongation zone grow to 135 μm in 4 hours, before entering the mature zone at their maximum size [32].

### 1.1.2   Auxin Influx and Efflux

The PIN-FORMED (PIN) family of auxin transporters is responsible for mediating auxin efflux within the growing root [15]. The polarized distribution of PIN proteins within each cell, including PIN1, PIN2, PIN3, PIN4, and PIN7, is instrumental in producing a stable auxin gradient [35]. The amount of PIN transporters on a cell membrane is mediated by intracellular recycling [3, 24]. Auxin has been shown to inhibit this process [24], which increases the amount of PIN on the membrane. Conversely, BR promotes PIN recycling through direct transcriptional regulation [10] and indirectly through CLASP [3].

Auxin influx is determined by the nonpolar AUX/LAX family of transporters [30] as well as passive diffusion through the cell membrane. Members of the AUX/LAX transporter family perform similar functions but are located in different regions of the cell. For instance, AUX1 is concentrated in the border tissue [29], while LAX2 and LAX3 are located in the vascular tissue and columella [30]. Together, the PIN efflux transporters nad AUX/LAX influx transporters produce a distinct auxin gradient within the cell [4]. In healthy roots, this gradient is characterized by an auxin maximum at the QC, gradually decreasing auxin levels up through the vascular tissue, and low auxin levels in the border tissue [9].

### 1.1.3 PLETHORA

When exposed to high concentrations of auxin for a long period of time, *Arabidopsis* roots experience an increase in PLETHORA transcription factors (PLT) [17]. PLT is known to upregulate the transcription of the YUCCA3 gene, which increases auxin biosynthesis. Additionally, PLT modulates cell elongation and differentiation on a much longer time scale than auxin. This allows the root to respond quickly to environmental stimuli via auxin redistribution, while maintaining stable zonation due to transcriptional regulation of PLT [17].

### 1.1.4 Cytokinin

Cytokinin is an important phytohormone with many important functions in the gorowing root. It has been shown to inhibit auxin flow through the vascular and columella tissue by restricting the expression of PIN1, PIN3, and PIN7 [12] via the SHY2 protein and ARR1/ARR12 signalling pathways. Additionally, cytokinin promotes the auxin inhibiting GH3 protein [6] and upregulates cell differentiation [12].

### 1.1.5 Brassinosteroid, CLASP, and Microtubules

Brassinosteroid (BR) is an essential part of the hormone network in *Arabidopsis* roots, and has shown to promote both longitudinal and radial growth in a spatiotemporal manner [1]. Unlike auxin, BR is not transported over long distances through the vascular tissue, but can diffuse locally [34]. Each cell synthesizes its own BR, based on a gradient of signalling enzymes that reaches a maximum in the elongation zone [34]. In the vascular tissue, BR has been shown to interact antagonististically with auxin in order to maintain the division and elongation zones [5]. Notably, BR is also known to promote auxin biosynthesis in the epidermis, which unveils a context-specific relationship between BR and auxin [33]. BR also indirectly influences auxin through transcriptional regulations of the PIN2 and PIN4 transporters [10].

Microtubules (MTs) are bundles of cellulose that form on the outside of cells. Depending on their orientation, MTs can restrict or induce growth in the cell [2]. Transverse MT arrays promote due to their limited resistance along the axis of growth. On the other hand, transfacial bundles created by the protein CLASP will restrict growth in all directions. BR has been shown

to inhibit CLASP and thus promote growth [25]. However, CLASP also upregulates the BR receptor BRI1, which creates a stable positive-negative feedback loop [25]. Additionally, CLASP has been shown to promote the recycling of PIN2 [3], but further research is needed to determine the complete crosstalk between CLASP and auxin.

## 1.2 Static Auxin Models

### 1.2.1 Modelling Frameworks

Two-dimensional models of auxin in the root can generally be divided into two categories. The first type of models approximate auxin flow through the root by discretizing advection-diffusion equations on a lattice, adding extra terms to denote cell walls and PIN mediated transport [6,9,22,23,28]. Due to the inherent restrictions of lattices, these models assume cells are rectangular [9, 22, 23] or approximate a realistic cell shape using simple curves [6, 28]. As an alternative, some researchers have chosen to model cells as polygons, representing the root as set of vertexes and edges based on images of *in vivo* roots [4]. More recent research [20, 21] has used this framework to perform further modelling studies.

### 1.2.2 Auxin Efflux and Influx

As discussed previously, auxin efflux is primarily mediated by PIN-family auxin transporters (PIN). Almost all modelling studies involving auxin incorporate PIN in some way, with varying levels of sophistication. The simplest implementations of PIN is to prescribe a static gradient which replicates the distributions observed *in vivo* [4, 9, 20, 21]. However, other models have implemented dynamic PIN distributions based on cytokinin [6, 22, 23, 28]. Notably,the is insufficient research exploring the effects of BR on PIN2 [3] within current models.

Auxin influx is regulated by the AUX1/LAX family of transporters. Some models opt to ignore these transporters outright, instead opting for a constant auxin influx accross all cells [9]. Other models prescribe AUX1/LAX transporters [4, 20, 21], which are deposited uniformly around the cell membrane. Some models have explored crosstalk between AUX1/LAX transporters and other hormones including auxin [28] and ethylene [22].

### 1.2.3 Background Diffusion

TBD

### 1.2.4 PLETHORA Transcription Factors

TBD

### 1.2.5 Cytokinin

TBD

### 1.2.6 Ethylene

TBD

## 1.3 Growing Root Models

Limited studies have attempted to model hormone gradients within growing roots [9,17,23,28] and these studies have applied relatively simplistic descriptions of cell and tissue growth [26]. Other studies have built highly accurate models of root growth using vertex-element models [7,8], but have not yet incorporated auxin dynamics. All models of root growth face the problem of *symplastic growth*, which refers to the idea that cells are unable to move independently of one another because they share a cell wall with their neighbours [13]. This implies that any two cells equidistant from the root tip must be growing at precisely the same rate, at all times [13]. Due to the fact that the distributions of auxin and other hormones are spatially regulated in both the transverse and longitudinal direction, defining a growth function that is constant along the horizontal axis is challenging. Grieneisen et al. [9] tackle this problem using a cellular potts model that restricts growth to the longitudinal direction to prevent cell sliding. The work of Mähönen et al. [17], later built on by Salvi et al. [28], instead determine growth by taking an average of the growth factors in all vascular cells and applying it to the entire row. Cells "grow" via the addition of a new row of simulation points to the lattice. Models of growing roots find strong evidence that root growth affects hormone dynamics [26], making solving the problem of modelling symplastic growth an important issue for further study.

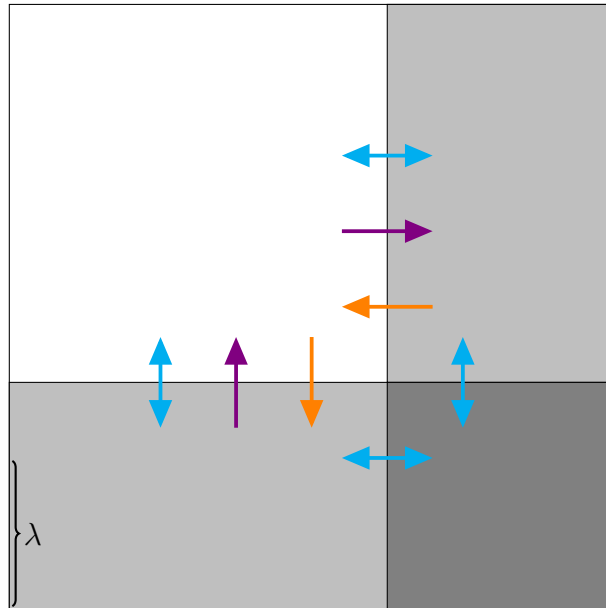# Chapter 2

# Model

## 2.1 Model

### 2.1.1 Root Geometry

This paper uses a vertex-based model. Edges and vertices form the cell wall, and the area enclosed by these edges represent the cytoplasm. Because the cell wall has a different diffusion rate than the cytoplasm [14], all of the vertexes, edges, and interior regions contain hormones.

## 2.2 Growth

## 2.3 Division

# Chapter 3

# Results

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# Appendix A

# Supplementary Materials

## A.1   Model Details

## A.2   Parameter Values