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Canalization-based vein formation in a growing leaf

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HIGHLIGHTS

- We study vein pattern formation in a growing leaf tissue.
- The canalization hypothesis is adopted as a chemical model for auxin flow.
- Tissue growth is described by a vertex dynamics model.
- Independent coupling of the two models cannot reproduce normal patterns.
- Cell mechanical property and growth rate need to be modified according to auxin flux.

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ABSTRACT

Vein formation is an important process in plant leaf development. The phytohormone auxin is known as the most important molecule for the control of venation patterning; and the canalization model, in which cells experiencing higher auxin flux differentiate into specific cells for auxin transportation, is widely accepted. To date, several mathematical models based on the canalization hypothesis have been proposed that have succeeded in reproducing vein patterns similar to those observed in actual leaves. However, most previous studies focused on patterning in fixed domains, and, in a few exceptional studies, limited tissue growth – such as cell proliferation at leaf margins and small deformations without large changes in cell number – were dealt with. Considering that, in actual leaf development, venation patterning occurs in an exponentially growing tissue, whether the canalization hypothesis still applies is an important issue to be addressed. In this study, we first show through a pilot simulation that the coupling of chemical dynamics for canalization and tissue growth as independent models cannot reproduce normal venation patterning. We then examine conditions sufficient for achieving normal patterning in a growing leaf by introducing various constraints on chemical dynamics, tissue growth, and cell mechanics; in doing so, we found that auxin flux- or differentiation-dependent modification of the cell cycle and elasticity of cell edges are essential. The predictions given by our simulation study will serve as guideposts in experiments aimed at finding the key factors for achieving normal venation patterning in developing plant leaves.

1. Introduction

The vascular system of plants, composed of phloem and xylem, which transport the products of photosynthesis and both water and dissolved minerals, respectively, plays an important role in survival and development (Evert and Esau, 2006). In plant leaves, vascular cells differentiate from procambial cells, which differentiate from undifferentiated meristematic cells (Steeves and Sussex, 1989; Baima et al., 2001). The phytohormone auxin is known to be the most important molecule for the control of differentiation of meristematic cells into procambial cells (Jacobs, 1952). There exists a net-like pattern of auxin transport in developing leaf tissue that acts as a pre-pattern for the differentiation of procambial cells (Uggla et al., 1996; Sieburth, 1999; Avsian-Kretchmer et al., 2002; Aloni et al., 2003; Fukuda, 2004). The polarity of auxin flow is regulated by the carrier protein PIN1 (Rubery and Sheldrake, 1974; Goldsmith, 1977; Morris, 2000) which is localized to a specific edge of cells and promotes the pumping of auxin molecules outside cells. Inhibition of PIN1 causes a dramatic change in leaf venation patterning (Galweiler et al., 1998; Mattsson et al., 1999; Berleth et al., 2000).

A widely-accepted hypothesis for the mechanism of vein pattern formation by auxin transport is canalization (Sachs, 1975,

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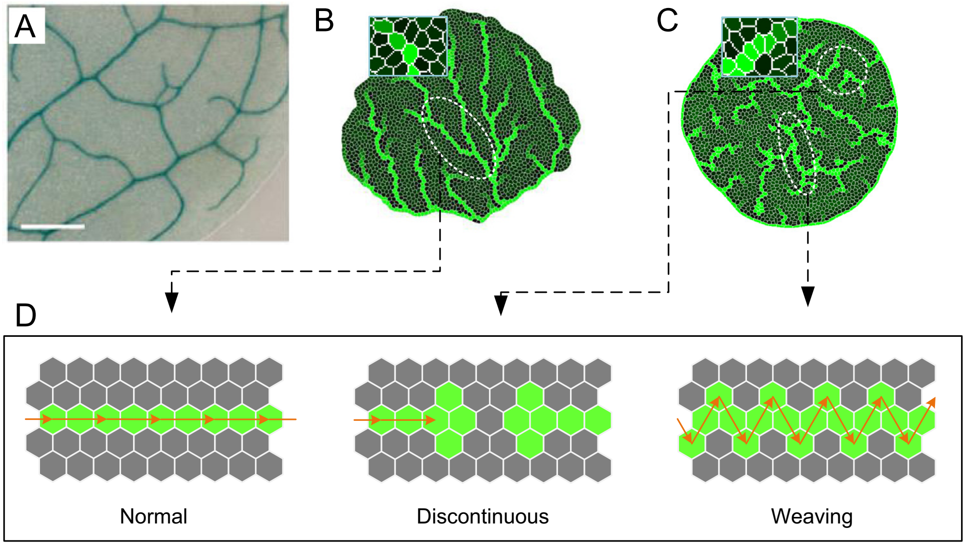

Fig. 1. Pilot simulations; independently combining chemical dynamics for canalization and tissue growth never reproduces normal venation patterning in a growing domain: (A) venation of an *Arabidopsis* leaf. Blue lines are vein cells (image reproduced with permission from Alonso-Peral et al., 2006). Scale bar, 250 μm. (B) A vein pattern obtained by numerical simulation in a fixed polygonal lattice, and (C) in a growing lattice (see Section 2 for details of mathematical modeling and the simulation procedure). In both simulations, the same parameters for chemical dynamics were used. The color of each cell indicates the auxin concentration relative to the average over all cells in each simulation. Lighter green corresponds to higher auxin concentration. (D) Simplified diagrams of normal and abnormal vein patterns. The abnormal pattern is due to the discontinuity and straightness problems. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

1981, 1991). In this hypothesis, cells experiencing a higher auxin flux than their neighbors become specialized for auxin transport and turn into auxin sinks (Sachs, 1991). The first mathematical model based on this scenario was proposed by Mitchison (1980a, b, 1981). In his model, auxin source and sink cells are placed at opposite sides of a fixed rectangular lattice, and asymmetric diffusion between cells, corresponding to polarized auxin transport, was introduced. He showed with this model that a laminar flow of auxin from source to sink first appears and that by adding a small perturbation to the system, a preferential auxin transport path is formed. However, in his model, the auxin concentrations in the cells forming the preferential path is lower than that in surrounding cells, which is inconsistent with the observation of higher auxin concentrations in vein cells.

Feugier et al. (2005) proposed an extended model that included the carrier protein PIN1. In that model, auxin is produced in all cells in a fixed hexagonal lattice and flows out of the lattice through a sink cell. Each cell has a certain fixed amount of carrier proteins which is assumed to localize to cell edges in response to the auxin flux. Since the carrier protein localizes to cell edges through which auxin flux is higher, auxin molecules pass more easily through those edges. Preferential paths of auxin emerged which showed higher auxin concentration than in surrounding cells as observed in experiments. The authors concluded that this positive feedback between carrier protein localization and auxin flux is a fundamental mechanism of venation patterning.

Thus far, several mathematical models based on the canalization mechanism have been proposed and have succeeded in reproducing branching vein patterns (Rolland-Lagan and Prusinkiewicz, 2005; Bayer et al., 2009; Walker et al., 2013) or reticulate vein networks (Fujita and Mochizuki, 2006; Feugier and Iwasa, 2006). However, most of these models focused on patterning in a fixed domain. The exceptions are the studies by Fujita and Mochizuki. (2006) and Wabnik et al. (2010), which dealt with limited tissue growth, e.g., cell proliferation at leaf margins or small deformations without large changes in cell number. Nevertheless, in actual leaf development, the venation patterns are gradually formed in dynamically growing tissues (Scarpella et al., 2006; Alonso-Peral et al., 2006; Sawchuk et al., 2013). Thus, it is natural to ask whether the canalization hypothesis still applies in dynamically growing leaf tissue.

In a pilot study, we performed simulations where a previously proposed canalization model is simply combined with tissue growth simulation (see Section 2 for details on modeling and simulation). As shown in Fig. 1, when the chemical dynamics governing the canalization mechanism is independent of tissue growth dynamics and cell mechanics, normal patterns are not realized at least for parameter ranges for which normal venation patterning is achieved in the absence of tissue growth (see Section 2.3 for details on parameter values). The anomalous patterns result from the fact that, due to dynamic change in the lengths of cell edges and the spatial arrangement of cells through tissue growth, auxin flow is interrupted (discontinuity problem) and tends to weave (straightness problem) (Fig. 1D), while in actual plant leaves, veins are continuous (almost no interruption) and quite straight (Fig. 1A).

In this study, motivated by our pilot simulations, we examine conditions sufficient for achieving normal venation patterns in growing leaf tissue by introducing various constraints on chemical dynamics, tissue growth, and cell mechanics. The effects of these constraints on the pattern are quantitatively evaluated using indexes of discontinuity and straightness of auxin flow. We found that, to achieve normal venation patterning, tissue growth dynamics and cellular mechanics need to change according to auxin flux or differentiation state. We expect that our results will provide a guidepost for experimental studies aimed at elucidating the fundamental mechanisms of venation patterning in developing plant leaves.

2. Model

2.1. Leaf tissue used in this study

We study here vascular development in the *Arabidopsis* leaf. We focus on vein formation in intercostal areas (IAs), which are sections enclosed by the main veins of the leaf (Fig. 2). IAs are created about 4 days after germination (DAG) as a result of the elongation of secondary veins and the formation of loops with the main vein. While the spatial patterns of the main and secondary veins are quite