Apical dominance models can generate basipetal patterns of bud activation

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An important aspect of plant development is the order in which lateral buds are activated to produce branches. This order may manifest itself, for example, in the gradient of branch lengths, or in the flowering sequence. One progression type is acropetal, with the lateral buds activated from the bottom up. Other plants exhibit a basipetal sequence of bud activation, with a downward progression of branch development. More complex, mixed sequences also occur, with the activation sequence converging towards or diverging away from the central part of the shoot axis. The acropetal progression can be simply explained by postulating that lateral buds are activated in the same order in which they were created (possibly with some delay). However, basipetal progression eludes such a simple explanation, raising the question of what mechanisms may be responsible. As details of the underlying signaling processes, and their interplay with development, are difficult to observe directly, simulation models are helpful in integrating diverse experimental data, in highlighting the dynamic relations between local processes and the resulting emergent structures, and in assessing plausibility of alternative hypotheses.

Basipetal activation progression is likely related to apical dominance. The hypothesis is that the shoot apical meristem in the vegetative state has a strong inhibitory influence on the lateral buds below, which is lifted upon the transition of the apex to the flowering state. This information propagates down the stem, causing gradual activation of the lateral buds. The inhibitory signal may be auxin, produced by the shoot apex and actively transported down the plant [Thimann and Skoog 1933]. The timing of activation of each successive bud might then reflect the speed with which the wave of auxin depletion propagates down the stem after the transition to flowering.

Although this "depletion-wave" model is capable of generating branching sequences of some plants found in nature [Lindenmayer 1984, Janssen and Lindenmayer 1987, Prusinkiewicz and Lindenmayer 1990], two questions can be raised. First, it is not clear how this model could account for the activation sequences of buds within rosettes. Here extremely short internodes should lead to almost simultaneous activation of lateral buds, yet in Arabidopsis, for example, a basipetal sequence is observed in the rosette in spite of the short internodes [Stirnberg et al. 1999, Figure 1A]. Second, the depletion-wave model does not take into consideration contributions of the lateral branches to the auxin flow in the stem, contrary to experimental data [Morris 1977].

To address these problems, we propose an alternative conceptual model of bud activation. Lifting of apical dominance due to floral transition of the main apex activates the topmost lateral bud, as in the wave model. At this point, the active lateral bud becomes a source of auxin that inhibits more basal buds, until its own switch to flowering. This in turn activates the next lateral bud, resulting in a mechanism in which the inhibitory influence is relayed from one active bud to the next.

To test the plausibility of this conceptual model, we developed a sequence of simulation models. Model 1 captures the essence of the relay process. A sequence of metamers, each

associated with an axillary bud, is created by the apex with a constant plastochron. Auxin produced in the apex is transported basipetally through these metamers, and is drained from the basal metamer by the root. Furthermore, the auxin is subject to decay in each metamer. Transition to flowering takes place after a user-defined number of metamers have been produced, and decreases the production of auxin. Activation of the lateral bud is controlled locally within each metamer, by the level of auxin falling below a specified threshold. Upon activation, a lateral apex produces auxin, which is transported into the main stem. As for the main apex, the lateral apex switches to the flowering state after a delay. As expected, a basipetal sequence of lateral bud activation and flowering emerges due to the relay effect. Interestingly, if the transition to flowering in the main apex is delayed, resulting in a larger number of metamers, the model produces a convergent activation sequence. This is due to the gradual decrease of auxin concentration in the basal direction due to auxin decay. As the distance between the apex and the basal metamers increases due to growth, auxin concentration in the basal metamers falls below the threshold for bud inhibition. This relation between metamer number and the pattern of bud activation is a robust emergent property of the model, and coincides with the change in the progression of activation in Arabidopsis plants grown in long-day (15 metamers) vs. short-day conditions (>30 metamers) [Stirnberg et al. 1999]. Furthermore, due to the residual production of auxin by the floral apices, a zone in which buds are never activated may emerge in the central part of the stem, as it does in Arabidopsis plants grown under short-day conditions.

Model 1 confirms the plausibility of the relay concept in regulation of bud activation. However, experimental data with radiolabelled auxin show that auxin transported from the main apex through the stem does not pass in the vicinity of the dormant buds, and does not enter them [Morris 1977]. This raises the question of how the auxin signal is conveyed to the bud. A widely held view is that auxin acts on the lateral bud indirectly, through the intermediacy of another hormone, cytokinin, which can move freely between the stem and the bud [Cline 1991]. It is known that cytokinin is synthesized in the root and in the stem, at rates negatively regulated by auxin [Li et al. 1995, Nordstrom et al. 2004, Tanaka et al. 2006], and directly promotes bud growth [Cline 1991]. We tested the plausibility of this intermediate-signal hypothesis by constructing Model 2, which incorporates the acropetal flow of cytokinin and the regulation of cytokinin synthesis by auxin. Bud activation is now triggered by the level of cytokinin in the bud exceeding a predefined threshold. Simulation results confirm that the relay mechanism can produce the observed activation patterns under these conditions as well.

The intermediacy of a second hormone, however, is not the only possible explanation for the action of basipetally transported auxin on lateral buds. Another possibility is based on two assumptions: that lateral buds remain dormant until they can export locally produced auxin, and that the main stem has a limited capacity for auxin transport. Thus, lateral buds compete with the main apex for limited auxin transport capacity of the main stem. As long as the main apex is active, the auxin it produces appropriates the entire transport capacity of the stem. After the switch to flowering, the auxin depletion wave relinquishes transport capacity, allowing for auxin transport from the most apical lateral bud. This triggers its activation and restores auxin flow in the stem. By the same mechanism, the subsequent switch to flowering of this bud triggers activation of the next one, and the relay progresses. We call this model the “traffic intersection model”, since it resembles the situation on a highway, where vehicles from the subsidiaries can only join the traffic if the carrying capacity of the highway is not fully utilized.

We tested the plausibility of this concept using Model 3. To account for the interplay between different streams of auxin flow (from the main apex vs. from the lateral buds), we used a more detailed model of auxin transport than in Models 1 and 2. Similar to the canalization model [Sachs 1981], we assume that auxin flux between adjacent metamers is a combination of diffusion and polar transport. Polar transport is related to auxin flux in a feedback loop, such that increased flux promotes more efficient polar transport in the direction of the flux. However, polar transport is capped, thus limiting the maximum auxin transport capacity. With

properly chosen parameters, diffusive transport of auxin from the apex towards the root rapidly triggers a saturated polar transport stream in the main stem. The resulting relatively high concentration of auxin in the stem limits diffusion from the lateral buds, thus inhibiting polar transport form the buds. The situation changes when the main apex switches from the vegetative to the floral state. This reduces auxin concentration in the stem. The diffusive flux from the lateral buds increases, triggering the establishment of polar transport into the stem. The resulting auxin outflux activates the buds. As in previous relay-based models, this process begins with the topmost lateral bud and proceeds downwards, with consecutive lateral buds using, then relinquishing, the carrying capacity of the stem in succession (Figure 1).

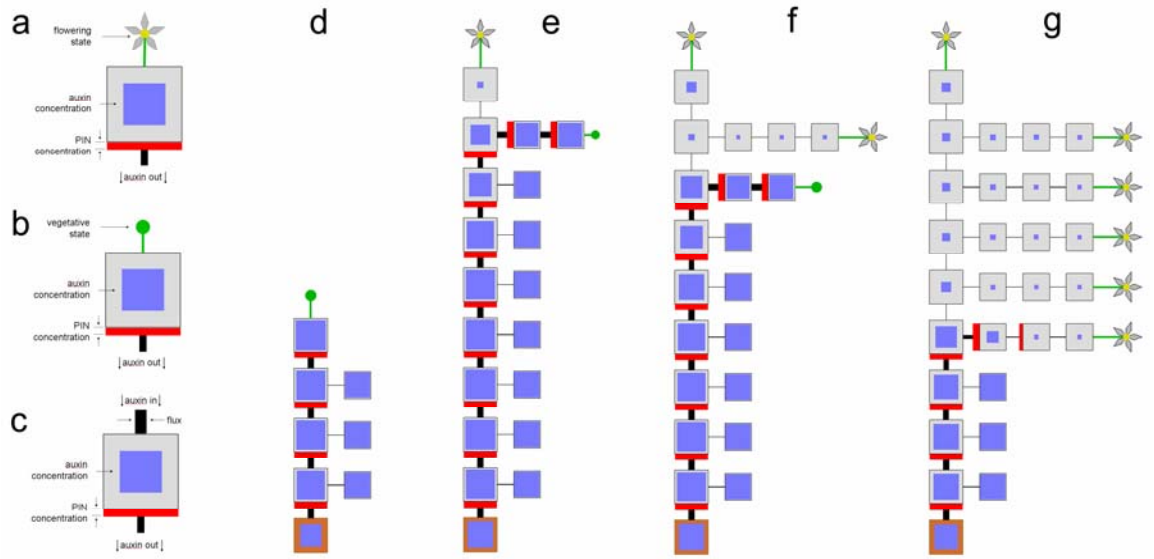

Figure 1. Illustration of Model 3. a-c) Schematic representations of an apex in the flowering and vegetative state. b) Schematic representation of a metamer. d-g) Selected stages of the simulation. The simulation begins with the main apex creating a sequence of metamers with the associated lateral buds. The flow of auxin from the apex saturates auxin transport capacity in the shoot (d). Upon transition to flowering, production of auxin in the main apex decreases. The resulting excess transport capacity in the stem enables auxin efflux from the topmost lateral bud, which results in its activation. Auxin produced by this bud re-saturates the stem (e). After transition of the topmost bud to the flowering state, the next lateral bud becomes activated (f). The resulting relay process continues (g) until all buds become activated.

There are several conceptually attractive features of this model. First, it explains the basipetal activation sequence of lateral buds without invoking additional signals. Next, the model can explain the phenotype of the Arabidopsis *max* mutants, where increased branching is associated with increased auxin transport capacity in the stem [Bennett et al. 2006]. Finally, the model integrates several aspects of auxin biology; in particular, in relates apical dominance and activation progression to canalization mechanisms.

An important aspect of canalization, however, is not only the feedback between auxin flow and cell or module polarization, but also the convergence of auxin flow into focused streams: canals, precursors of vascular differentiation. In the case of lateral buds, vascular connections may be formed concurrently with, and indeed as an integral part of, increased auxin outflow from the buds [Grbic and Bleeker 2000, Figure 2]. To test whether Model 3 is compatible with such behavior, we constructed a schematic model of canalization at tissue level, modified from [Rolland-Lagan and Prusinkiewicz 2005]. The resulting Model 4 expresses cell polarization in terms of the allocation of efflux carriers (PIN proteins) from a given pool to specific faces of the cell according to net auxin efflux [Feugier et al. 2005]. A polarization exceeding a predefined threshold is locked, simulating vascular differentiation. In the sample simulation illustrated in

Figure 2, the cellular grid is constrained to a shape representing a longitudinal section of the stem with two buds (b). Following the placement of an auxin source at the top of the main segment, a vascular strand running through the segment emerges (c). Subsequent placement of auxin sources in the two buds (d) does not trigger formation of lateral vasculature until the auxin source at the top of the main stem is removed. The resulting decrease of auxin concentration in the main vasculature then triggers the formation of a vein connecting the higher bud to the central vasculature (e). When the source of auxin associated with this bud is removed, a similar process occurs in the lower bud (f). This confirms that the "traffic intersection" model of bud activation (Model 3) is consistent with processes that take place at the tissue level according to the canalization paradigm (Model 4).

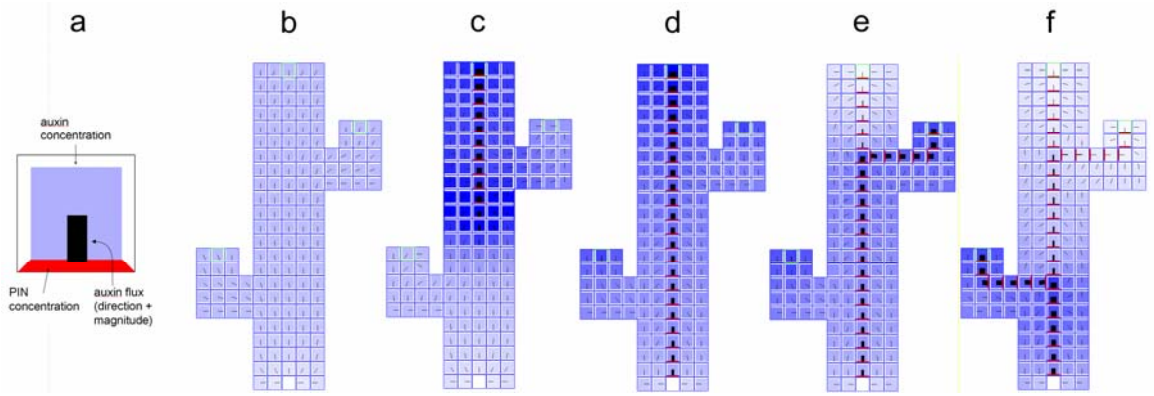

Figure 2. Illustration of Model 4. a) Iconic representation of a cell. b-f) Selected stages of the simulation. Detailed explanation in the text.

In conclusion, we have shown that relay models are capable of forming basipetal and convergent activation patterns in a manner qualitatively consistent with experimental data. Variations of the basic model (Model 1) confirm the plausibility of postulated molecular-level mechanisms: mediation of auxin action by cytokinin (Model 2), and competition for the limited auxin-carrying capacity of the stem (Models 3 and 4). These mechanisms are not mutually exclusive, and can operate in concert. Our models provide a basis for further simulation studies of processes controlling activation patterns, such as the effect of mutations and manipulations of auxin distribution and transport on the activation patterns. Another open problem, challenging from both a conceptual and modeling perspectives, is the interplay between hormones and resources in bud activation.

Materials and methods. Architectural-level models (1-3) were specified in the L-system-based L+C language [Karwowski and Prusinkiewicz 2003]. The tissue-level Model 4 was specified in the vv modeling language [Smith et al. 2004]. All models were implemented in the L-studio/vlab environment [Prusinkiewicz 2004].

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