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Auxin canalization: From speculative models toward molecular players

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Abstract

Among the most fascinated properties of the plant hormone auxin is its ability to promote formation of its own directional transport routes. These gradually narrowing auxin channels form from the auxin source toward the sink and involve coordinated, collective polarization of individual cells. Once established, the channels provide positional information, along which new vascular strands form, for example, during organogenesis, regeneration, or leave venation. The main prerequisite of this still mysterious auxin canalization mechanism is a feedback between auxin signaling and its directional transport. This is manifested by auxin-induced re-arrangements of polar, subcellular localization of PIN-FORMED (PIN) auxin exporters. Immanent open questions relate to how position of auxin source and sink as well as tissue context are sensed and translated into tissue polarization and how cells communicate to polarize coordinately. Recently, identification of the first molecular players opens new avenues into molecular studies of this intriguing example of self-organizing plant development.

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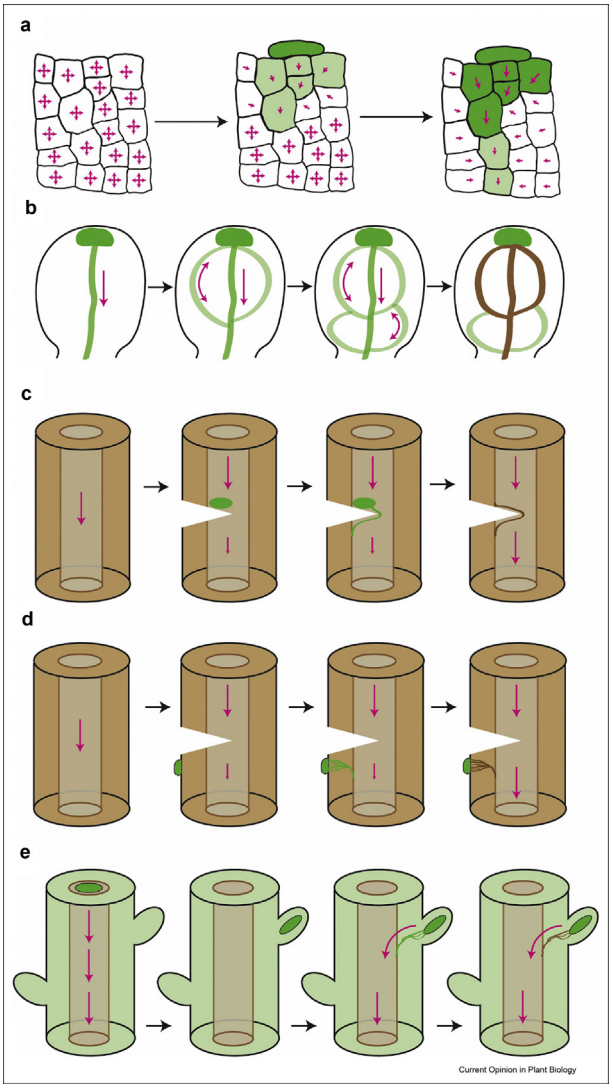
Auxin canalization and its developmental roles

The plant vascular tissue forms highly ordered and complex patterns, which secure exchange of nutrients and assimilate between organs. Since decades, research works have been puzzled how the vascular pattern spontaneously, flexibly but predictably arise in the different developmental contexts, for example, connecting newly arising organs or lateral buds released from dormancy with pre-existing vascular network, around wounds, which interrupted vasculature, or during formation of the intricate patterns of leave veins. Early experiments showed that the phytohormone auxin alone is the major and sufficient signal inducing formation and differentiation of vascular strands in a variety of systems [1]. Conceptual breakthrough from this visionary work of Tsvi Sachs led to formulation of the so-called canalization hypothesis proposing that auxin, by a feedback regulation of its own transport, induces formation of self-organizing channels. They start initially from a broad field of auxin-transporting cells and gradually narrow until well-defined channels with high auxin transport capacity arise (Figure 1). They form from an auxin source toward the sink, and during this process, the individual cells coordinately polarize by integrating the position of auxin source and sink, tissue context, and status of neighboring cells [2,3].

Directional auxin flow is facilitated mainly by PIN-FORMED (PIN) auxin exporters, whose subcellular polar localization determines directionality of the cell-to-cell auxin flow [4,5]. As predicted by canalization hypothesis, PIN polarity can be indeed rearranged in response to auxin [6–8] and, accordingly, gradually narrowing, PIN expressing, and polarizing auxin channels have been observed to precede most cases of vasculature formation [8,9,10,11,12,13]. In others, such as vasculature reconnecting during grafting, a link between PIN-dependent auxin transport and vascular reconnection has not been established [14].

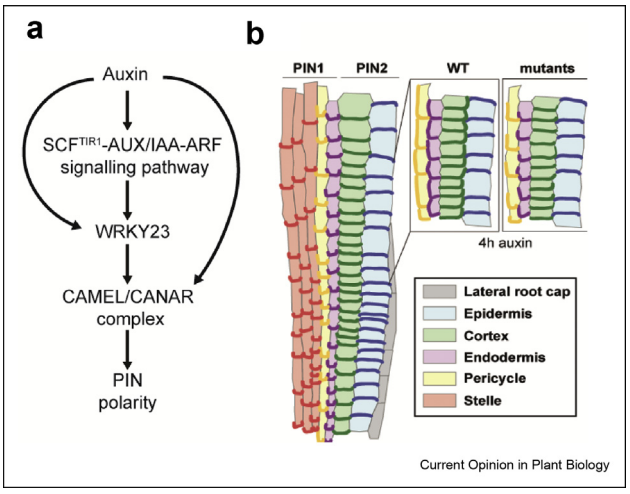
A simple model for visualization of auxin effect on PIN polarity is the *Arabidopsis thaliana* root meristem, where auxin treatment leads to tissue-specific PIN relocalization from the basal to inner- (for PIN1) or outer- (for PIN2) lateral sides (Figure 2). This is a reproducible robust effect, nonetheless, with an unclear physiological significance [6,7,15]. Importantly, all mutants showing defects in this auxin-induced PIN relocation also show defective leave venation, vasculature regeneration, and vasculature formation from the local auxin source [6,7,10,15,16,17]; processes to which auxin canalization is essential.

Figure 1



Auxin canalization in plant development. (**a**) Model of canalization: Auxin from a source (represented in dark green) polarizes originally homogeneous cells to initiate directional transport of auxin (marked by the magenta arrow) away from its source. The self-organizing property of auxin transport allows canalizing auxin from an initially broad domain into a narrow channel with high auxin-transporting capacity. (**b**) Leaf venation: The auxin maximum in the leaf tip drives the induction of auxin-transporting channels, demarcating future positions of vasculature. (**c**) Regeneration after wounding: Interruption of stem vasculature results in local auxin accumulation above the wound. Auxin is canalized around the wound to reconnect the vasculature. (**d**) External application of auxin below the wound triggers auxin channel formation followed by vasculature connecting the auxin source with the pre-existing vasculature. (**e**) The shoot apex is a well-known source of auxin which keeps lateral buds inhibited. Once the apex is removed, the closest lateral bud is released from the inhibition and becomes a new dominant auxin source. Auxin-induced vasculature differentiation connects the lateral bud to the pre-existing stem vasculature. Green: auxin accumulation, auxin channels; magenta arrows: auxin flow; brown: differentiated vasculature.

Figure 2



Auxin-induced PIN repolarization in root meristem. (**a**) The known molecular players of auxin feedback on PIN polarity. (**b**) Prolonged auxin treatment repolarizes PIN1 in the pericycle/endodermis from the basal to inner lateral side of the cells, whereas PIN2 in cortex undergoes a basal to outer-lateral polarity shift. In the mutants with defective auxin feedback on PIN polarity, auxin-induced repolarization is reduced.

Thus, the positive feedback regulation between auxin and polar PIN localization appears to be a crucial part of the auxin canalization mechanism, despite the most molecular components of this process await identification, and it is likely that also other transport systems such as auxin influx are involved [18].

Mechanistic models of canalization

Even with a demonstration that auxin indeed induces coordinated polarization of PIN transporters, the underlying mechanism remains mysterious. How do the individual cells perceive the often distantly positioned auxin source and by which mechanism do they place PINs on the appropriate cell side? Is it the auxin flow through the cell, which polarizes PINs, or does the cell places PINs toward the neighbor with the lowest auxin levels? Based on modeling, both these scenarios can generate a correct pattern of PIN polarizations and auxin channels [2,19]; nonetheless, it is hard to envision a biologically plausible mechanism for measuring either auxin flux or its concentration in all neighboring cells. Two other conceivable mechanisms were proposed how polarities can be propagated from cell to cell: (i) auxin acting on cell growth and causing mechanical stresses transmitted via the cell wall to the neighbors [20]; or (ii) a combination of intracellular and extracellular auxin perception, which regulates PIN expression and endocytic recycling, respectively, and ultimately causes differential PIN

incidence at the opposite cell sides [21]. Variations of the latter model successfully predicted polarities and auxin accumulation patterns in embryogenesis [22,23].

Indeed, PINs are dynamically cycling between their polar domain at the plasma membrane and endosomal compartments allowing for rapid changes in PIN polarities. PIN endocytosis and limited lateral diffusion at the plasma membrane are required for PIN polarity (re)establishment after cell division and for its maintenance [24,25]. The PIN recycling and partially endocytosis as well are mediated by ADP-ribosylation factor guanine−nucleotide exchange factor trafficking regulators [26,27]. The *gnom* mutant defective in a major by ADP-ribosylation factor guanine−nucleotide exchange factors fails to form continuous PIN1-positive channels in leaves leading to disorganized vasculature [13].

The canalization model incorporated an auxin effect on PIN endocytosis as a key part of the feedback on PIN polarity [21] based on earlier experimental observations proposing that auxin inhibits PIN1 endocytosis [28]. Nonetheless, this does not apply for PIN2 [29], and more advanced techniques did not detect direct auxin effect on endocytosis at the plasma membrane but rather on PIN endocytic trafficking [30]. This auxin regulation presumably occurs by a so far uncharacterized auxin signaling mechanism inducing ultrafast phosphorylation of myosin XI proteins shown to be required for both auxin regulation of PIN trafficking and PIN polarity [preprint [31]].

Importantly, genetic analysis in *Arabidopsis* confirmed that PIN subcellular dynamics, PIN endocytosis, actin, and myosin function are all required for auxin canalization during *de novo* vasculature formation from the local auxin source and for vasculature regeneration after wounding [[16], preprint [31]].

Thus, despite the overall cellular mechanism of canalization remains still unclear; the auxin feedback on PIN polarity requires actin-myosin complex-dependent constitutive endocytic recycling of PIN proteins and its regulation by auxin-triggered phosphorylation. This constitutes a stepping stone for further elucidation of this mechanism.

Auxin input into canalization

One of the key questions in canalization concerns how auxin enters the process, that is, the upstream auxin perception and signaling mechanism. The mentioned canalization/polarization model assumes combination of both, intracellular and extracellular auxin perception [21].

Canonical transport inhibitor response 1/auxin-signaling f-box-mediated intracellular auxin perception

The best-characterized, predominantly, nuclear auxin signaling mechanism involves TRANSPORT INHIBITOR RESPONSE 1/AUXIN-SIGNALING F-BOX (TIR1/AFB) auxin receptors, auxin/indole-3-acetic acid transcriptional repressors, and AUXIN RESPONSE FACTOR transcription factors mediating transcriptional reprogramming [32]. This pathway mediates auxin regulation of PIN transcription in a tissue-specific manner [33]. Mutants in all these components show defects in auxin-induced PIN polarity changes in roots and in canalization-mediated vasculature formation, implying that this pathway also controls transcription or activity of regulators of PIN polarity and auxin canalization [6,7,10]. Transcriptional profiling identified a new molecular player downstream of this auxin signaling − a transcription factor WRKY DNA-BINDING PROTEIN 23, which is essential for auxin feedback on PIN polarity and canalization [7].

These observations revealed a role of TIR1/AFB transcriptional signaling in auxin feedback on PIN transcription and its action upstream of molecular components of PIN polarity.

Noncanonical, transmembrane kinase-mediated cell surface auxin signaling

The mentioned modeling approaches postulated also an essential role of auxin signaling initiated at the cell surface [21]. Furthermore, the ultrafast auxin-triggered phosphorylation of myosin XI, which is required for canalization, does not seem to depend on the canonical TIR1/AFB signaling [preprint [31]]. This collectively implies an existence of a noncanonical, cell surface auxin signaling important for canalization.

Candidate components for such auxin pathway are TRANSMEMBRANE KINASES (TMK1-4), which are leucine-rich repeat receptor-like kinases (LRR-RLKs), proposed to mediate auxin signaling at the plasma membrane. The *tmk1 tmk4* double mutant exhibits severe growth and developmental defects, some of which are indicative of perturbed auxin signaling [34,35], and the pathway was recently linked to auxin-regulated processes such as apical hook opening [36], root gravitropism [37], root and shoot growth [38,39], and lateral root formation [40]. Recently, TMK1 was shown to mediate auxin-induced phosphorylation of H^+-pumps in context of root and shoot growth [38,39]. Nonetheless, it remains to be determined, whether the TMK pathway mediates the overall auxin-triggered ultrafast phosphorylation response, including the phosphorylation of myosin XI, and whether it plays any role in canalization.

Even more pressing question reaching beyond the canalization topic is how TMK pathway perceives auxin; whether this occurs through its interactor — the controversial AUXIN BINDING PROTEIN1 (ABP1) [3,41]. In the verified *abp1* loss-of-function mutants, only minor developmental defects have been detected, whereas gain-of-function alleles show a much broad range of defects [42]. Nonetheless, the role of ABP1 in the ultrafast phosphoresponse or auxin canalization and vasculature regeneration has not been tested.

Thus, despite an involvement of noncanonical, cell surface auxin signaling in canalization has been envisioned; no supporting experimental results are yet available.

Canalization-related auxin-regulated malectin-type rlk- canalization-related rlk complex in coordination of pin1 polarization during auxin canalization

Not only that cellular mechanism of canalization remains hypothetical and mechanism(s) of auxin input is not clarified but also identity of most molecular components remains unknown. Recently, a transcriptome profiling downstream of the TIR1/AFB-WRKY DNA-BINDING PROTEIN 23 signaling module identified novel PIN polarity regulators — an LRR-RLK CANALIZATION-RELATED AUXIN-REGULATED MALECTIN-TYPE RLK (CAMEL) with its interactor CANALIZATION-RELATED RLK (CANAR) [15]. CAMEL and CANAR form a complex at the plasma membrane and execute effect on PIN polarity by interacting with and phosphorylating PIN proteins. In an absence of either CAMEL or CANAR functions, cells individually cannot polarize PINs in response to auxin and cannot coordinately orient PIN1 polarities into continuous auxin-transporting channels, thereby resulting in vasculature formation and regeneration abnormalities. A regulation of PIN polarity or activity by phosphorylation on overlapping sites is common [43] but CAMEL-targeted phosphosites in PIN1 loop appear to be unique and not shared by other known kinases. Phospho-mutated version of such PIN1 localizes apolarly, and it is insensitive to the auxin regulation, which leads to defective vasculature patterning, regeneration, and directional auxin transport as evidenced by '*pin1*-like' naked inflorescence phenotype. Given the weaker vasculature defects in *camel* or *canar* mutants, these phosphosites are most likely targeted also by other kinases in different developmental contexts [15].

It seems that CAMEL-CANAR module is yet another regulator of PIN polarity. Although other kinases such as PINOID or MITOGEN-ACTIVATED PROTEIN KINASEs control preferentially the apical/basal PIN polarity [44,45], CAMEL-CANAR seems to be rather specific for coordinated PIN polarity rearrangements during auxin canalization; this is also supported by the absence of any other obvious phenotype defects in the corresponding mutants. The imminent question for this mechanism relates to the nature of the extracellular ligand activating CAMEL-CANAR receptor complex and how this mechanism is linked to auxin input.

Conclusions

Despite extensive theoretical considerations as well as recent insights into cellular and molecular aspects of canalization, in particular, into auxin feedback on polarity of PIN auxin transporters, the major conceptual questions of canalization remain unanswered. How cells sense the position of auxin source and sink as well as tissue context to form defined auxin-transporting channels? By which mechanism individual cells correctly position PIN auxin transporters away from the auxin source? How cells propagate the polarities and how do they coordinate with each other? It seems that at least two signals need to be integrated by individual cells and translated into PIN polarity changes: (i) long-distance signal presumably by auxin gradient across the tissue, which gives a general source > sink direction; and (ii) unknown short-range signal used for coordination of neighboring cells. The latter is also supported by recent work showing that when auxin transport in leaves is genetically or pharmacologically inhibited, auxin retains its vein-patterning activity, although visibly disorganized [13]. This suggests existence of a new auxin-derived ligand which would serve as a positional cue for cells to coordinate their polarity and to canalize auxin [46,47].

A candidate for such a rapid short-range signal is Ca^{2+} because transient changes of cytoplasmic calcium ion concentration are essential for PIN polarity changes in root [48] or during shoot apical meristem development [49]. Indeed, auxin, via TIR1/AFB pathway, was shown to rapidly trigger cytosolic Ca^{2+} transients, possibly related to root growth regulation [50,51]. On the other hand, CAMEL and CANAR are LRR-RLKs, whose extracellular domains typically perceive external signals and translate them into cellular responses. The ligand for this pathway is unknown but may be related, for example, to secretory peptides or cell wall components. In any case, CAMEL-CANAR is a good candidate to participate in the short-range communication during canalization.

In animals, concentration gradients of morphogens can create astonishingly well-defined patterns such as zebra's stripes or cheetah's spots. The intercellular auxin gradient can transcriptionally control PIN and CAMEL-CANAR receptor complex and via its ultrafast phosphorylation target other processes such as myosin XI-dependent PIN trafficking, whereas extracellular ligand of CAMEL-CANAR may control PIN polarity also

via phosphorylation, together providing cells with global and local spatio-temporal information to coordinate for auxin channel formation. All these scenarios, while conceivable, are still speculative. Nevertheless, availability of the first specific components of canalization such as CAMEL and CANAR represents a good starting point for identification of further players and placing them into a less speculative mechanistic framework.

Further understanding of auxin canalization might be also accelerated by adoption of single-cell transcriptomics and proteomics. These approaches in PIN-expressing cells during auxin canalization in leaves can yield additional molecular players, which would be otherwise lost in noise of mixture of tissues using conventional methods.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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