Auxin Cell Biology in Plant Pattern Formation

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Auxin Cell Biology in Plant Pattern Formation

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Abstract

Auxin has always been implicated in a vast array of plant processes, but concomitant with a more detailed understanding of the cellular mechanisms underlying its biosynthesis, transport and perception, it has become increasingly clear that auxin has also instructive roles in plant pattern formation. Moreover, it turns out that in a multitude of instances, from early body axes establishment to organogenesis in shoot and root, plant tissue patterns owe their robust flexibility in part to feedback interactions involving auxin. Higher resolutions cell biology, molecular genetics and genomics as well as live imaging now are used together to define the parameters needed to generate more detailed and precise mathematical models of plant development.

Key words: auxin, plant development, *Arabidopsis*

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Auxin in self-organized plant development

Just like the other four classic phytohormones cytokinins, gibberellic acid, abscisic acid and ethylene (Leyser 1998; Estelle 2001; Gray 2004; Davies 2010), auxins are produced in a diversity of locations and have a broad spectrum of functions that affect virtually all organs of the plant (Teale et al. 2006; Chandler 2009; Zhao 2010). Although there are several natural forms of auxin, it is mainly represented in higher plants by indole-acetic acid (IAA) (Estelle 2001). Sites of intense IAA synthesis are located in the young shoot organs (Thimann 1977; Cheng et al. 2006, 2007), but IAA can also be turned into, or released from inactive forms (IAA conjugates) in a variety of locations (Van Staden 2006; Tivendale et al. 2014).

Auxin may be the most versatile of all the plant hormones, as it is implicated in the regulation of processes as diverse as cell expansion, cell division, apical dominance, gravitropism, phototropism, response to pathogens and abiotic stress (Mockaitis and Estelle 2004; Woodward and Bartel 2005; Kazan and Manners 2009; Davies 2010; Wang et al. 2010; Fu and Wang 2011). Some of these responses occur very rapidly and may not involve detectable changes in gene expression. However, during some developmental processes, auxin acts through the regulation of auxin-responsive genes (Boer et al. 2014). Examples of auxin-controlled developmental programs include, but are not limited to, patterning of the female gametophyte and the embryo, patterning of shoot and root meristems, or the organization of vascular tissues and stomata development, all of which have been portrayed in excellent recent summarizing articles (Sehra and Franks 2015; Ten Hove et al. 2015; Smith and Weijers 2015; Gallavotti 2013; Bennett et al. 2014; Kazan 2013; Sablowski 2013; Sawchuk and Scarpella 2013a; Balcerowicz et al. 2014).

In this review, we will focus on those instances of auxin mediated patterning in the shoot, where there is already considerable evidence for a crucial role of auxin in selecting cells in spots or lines as part of a self-regulatory process. Studies of such instances have revealed unexpected cell biological links between seemingly unrelated processes, like for example, phyllotaxis and leaf venation and have simultaneously opened up the perspective of a mathematical description of a self-regulatory system involving auxin and other plant hormones.

Auxin is not the only type of phytohormone involved in the regulation of developmental processes, but its complex framework of self-regulated transport and distribution is particularly intriguing as

part of self-organizing patterning itself (Leyser 2006; Grieneisen et al. 2007; Jeong et al. 2012). Although the question of whether auxin acts as a morphogen, is still a matter of debate (Peer et al. 2011), in processes like the positioning of leaf primordia around the shoot meristem (phyllotaxis), auxin seems to act as an instructive, as opposed to a permissive, patterning signal (Reinhardt et al. 2003; Bhatia et al. 2016).

The distribution and transport routes of auxin are more difficult to trace than one might expect, because neither auxin concentrations nor the transfer of auxin from cell to cell can be visualized directly, although new promising research tools are developing in form of auxin conjugated with fluorescent traces such as fluorescein and rhodamine isothiocyanate, conjugates that are shown to have auxin-like biological activity and transport (Sokołowska et al. 2014). Also, DII-VENUS a synthetic auxin sensitive marker which is rapidly degraded in response to auxin (Brunoud et al. 2012; Liao et al. 2015) proves to be a useful tool in detecting auxin changes although, to this end, the synthetic auxin-responsive promoter-reporter marker DR5 (Ulmasov et al. 1997; Sabatini et al. 1999; Benková et al. 2003; Friml et al. 2003) and its new variant (Liao et al. 2015) are widely accepted as a relatively unbiased indicator of auxin concentration. Similarly, transcriptional or translational fusions of members of the PIN-FORMED (PIN) family of auxin transport facilitators (Gälweiler et al. 1998; Steinmann et al. 1999; Wisniewska et al. 2006; Adamowski and Friml 2015) are used as markers for auxin transport routes at single-cell resolution (Scarpella et al. 2006; Marcos and Berleth 2014; Omelyanchuk et al. 2016). In combination, both types of markers have been able to draw a relatively consistent picture of auxin-based self-regulation in the patterning of diverse organs and are among the most used ones to test predictions from mathematical distribution models (Grieneisen et al. 2007; Grigolon et al. 2015; Boot et al. 2016).

The influence of auxin on patterning processes

Plant morphology is extremely diverse and even individuals within the same species have to retain an ability to continuously adapt their morphology to specific growth conditions. Despite this diversity, initiation of a new growth axis at a specific spot and the organization of this growth axis as an arrangement of concentric tissue layers are common motifs underlying highly diverse branching patterns (Capron et al. 2009; Jeong et al. 2012; Kierzkowski et al. 2013).

Within this context, highly flexible, yet precise patterning programs are needed for correct

specification and arrangement of tissues within organs and the entire body plan (Ten Hove et al. 2015). The genes responsible for encoding or interpreting positional cues have often been identified by diagnostic mutant phenotypes in *Arabidopsis*, which through the cell biological function of their gene products confirmed a role of auxin in patterning (Jeong et al. 2012; Vanneste and Friml 2012; van Dop et al. 2014; Ten Hove et al. 2015). These interpretations connected the wealth of earlier physiological data with subsequent genetic studies to explain how auxin, despite its vast spectrum of seemingly unrelated functions, can nevertheless have a decisive and specific impact on plant patterning (Sachs 1991; Berleth and Sachs 2001; Davies 2010).

Conceptual considerations of auxin-mediated patterning

Historically, a number of observations were seen as incompatible with a role of auxin in plant patterning (Bhalerao and Bennet 2003). Auxin appeared to be ubiquitous, transported in comparatively large amounts through mature vascular tissue, and few processes could be specifically initiated by the application of auxin (Davies 2010). However, the fact that a rather mundane and abundant molecule could have a highly specific and strictly regulated function in certain locations is not without precedent in plants or animals. Auxin may not have any patterning role when transported in large amounts through differentiated tissue, but small amounts can still impact patterning in young, meristematic tissue. One of these functions, the generation of continuous vascular strands in diverse configurations, were already evident in pioneering auxin-application experiments (Sachs 1975).

In a highly comprehensive approach Tsvi Sachs and collaborators, integrated large series of auxin application experiments with mathematical modelling to generate the auxin-canalization model for vascular patterning (Mitchison 1980; Sachs 1986). This model provided the first self-regulatory concept to explain the formation of files (lines) of cells in subepidermal tissue and, indirectly, the formation and internal organization of a new body axis (see below). What was missing in the model was a self-regulatory mechanism for the definition of concrete spots, for example, those to define the initiation sites of new axes. This void was filled by the observation that auxin application to shoot meristems can initiate formation of new lateral organs (Reinhardt et al. 2000). Further experiments involved the visualisation of expression and subcellular localisation of PIN1 as a marker of auxin flow (Reinhardt et al. 2003). Most importantly, it turned out that undisturbed organ initiation sites

were defined as locations of auxin convergence, specifically within the epidermis, which unlike sub-epidermal canalisation implies auxin transport against a concentration gradient. Figure 1 A and B summarizes the basic concepts of what is referred to as “up-the-gradient” or against the flow and “with-the-flux” or with the flow auxin patterning concepts, the first being confined to epidermal tissue. Because plant development is highly reiterative, those principles can be found as core mechanisms in a diversity of individual locations throughout the life-cycle of a plant (Capron et al. 2009; Bennet et al. 2014).

The de novo initiation of a body axis

Arabidopsis embryo is formed through a sequence of standardized cell divisions (Friml et al. 2003; Berleth et al. 2007; Izhaki and Bowman 2007; Capron et al. 2009; Petrásek and Friml 2009; van Dop et al. 2014; Ten Hove et al. 2015; Smith and Weijers 2015). The first division of the zygote is asymmetric, producing a smaller apical cell with dense cytoplasm and a larger basal cell with large vacuoles (Figure 2 A). Once the apical cell divides horizontally, it produces an upper and a lower tier of cells (Figure 2 B). Upper tier cells give rise to cotyledons and shoot apical meristem (SAM), the lower tier to the hypocotyl and to parts of the root apical meristem (RAM). Figure 2 provides a scheme of six embryonic stages, each defined by invariable cell divisions, which have been shown to be coupled with specific auxin distribution in *Arabidopsis* embryogenesis (Izhaki and Bowman 2007; Capron et al. 2009; Petrsek and Friml 2009; Wabnik et al. 2013).

The initiation of the apical-basal axis of the embryo becomes recognizable as parallel cell divisions within the lower tier of the dermatogen and globular embryo. This is immediately followed by the formation of the epidermis (protoderm), which is accompanied by specific auxin flux through the epidermis (Capron et al. 2009; Petrsek and Friml 2009; Figure 2 C, D). The newly established cell walls are oriented parallel to the apical-basal axis, generating narrow cells subsequently arranged in increasing numbers of parallel cell files. The next divisions generate even narrower cells in the centre of the lower tier, which will develop into the vascular tissue (stele) of the hypocotyl a site of intense auxin flow (Capron et al. 2009; Ten Hove et al. 2015) (Figure 2 E, F).

There is now abundant genetic evidence for the mechanisms through which this early stele anchors the patterning within surrounding ground tissue, the location of the RAM, the opposed position of the cotyledons, and through those the position of the SAM (Hardtke and Berleth 1998; Hamann et al.

2002; Szemenyei et al. 2008; Capron et al. 2009; Schlereth et al. 2010; Robert et al. 2013, 2015; Wendrich and Weijers 2013; Radoeva and Weijers 2014; Ten Hove et al. 2015). Therefore, it seems that an early auxin canalization event, by generating the embryonic stele, establishes the basis for the formation of a radially structured main body axis, and of two apical meristems at its ends (Petrásek and Friml 2009; Wabnik et al. 2013), (Figure 2, PIN1 and PIN7 distribution).

This interpretation is consistent with the robustness of the process to occur even within the variable dimensions of somatic embryos (Heidstra and Sabatini 2014) and with the fact that many mutations altering cell dimensions and other parameters do not obstruct it (Torres et al. 1994). In fact there are only few mutations that can entirely obstruct embryo axis formation without arresting embryo development, and conspicuously, all of these mutations interfere with either auxin synthesis (Cheng et al. 2007; Stepanova et al. 2008), transport (Friml et al. 2003) or auxin perception (Hardtke and Berleth 1998; Berleth et al. 2000). For example, mutations in a single *PIN* gene, *PIN1*, lead to cotyledon fusions that have also been observed in embryos treated with auxin transport inhibitors (Hadfi et al. 1998). Stronger auxin transport inhibition is associated with complete obstruction of embryo axis formation, phenocopied by mutations in the *Arabidopsis* *GNOM* (*GN*) gene (Steinmann et al. 1999; Geldner et al. 2003, 2004). This gene encodes a guanosyl-exchange factor, necessary for the activity of adenosyl-ribosylation factor, a RAS-like regulator, required for the organized and targeted transport of multiple PIN proteins to locations in plasma membrane. Therefore, only completely mislocalized PIN proteins in *gn* mutants lead to distortions of auxin transport severe enough to result in essentially non-polar embryos. Remarkably, this dramatic phenotype is not associated with other obvious types of cellular defects and *gn* cells can be cultured even without ever forming plant organs (Mayer et al. 1993; Richter et al. 2010). Not only disruption of auxin transport, but all interference with the auxin perception, in the form of loss-of-function mutations in the *MONOPTEROS/AUXIN RESPONSE FACTOR 5* (*MP/ARF5*) can occasionally lead to nearly unpolar embryos, which produce neither cotyledons nor roots (Przemeck et al. 1996; Hardtke and Berleth 1998; Hardtke et al. 2004). While this extreme phenotype is rather exceptional in *mp* single mutants, it is frequently associated with double mutations in *MP* and *NON-PHOTOTROPIC HYPOCOTYL4* (*NPH4*)/*ARF7*, the *ARF* most closely related to *MP* (Hardtke et al. 2004). Through the same distortion of auxin signal transduction, also gain-of-function mutations in *BODENLOS* (*BDL*)/*IAA12* lead to similar defects as *mp* loss-of-function mutations, since BDL functions as a negative regulator of MP activity (Hamann et al. 1999, 2002; Weijers et al. 2006; Lau et al. 2011). Lastly, auxin

synthesis has also been shown to be essential for proper establishment of the basal region of the embryo. Quadruple loss-of-function mutations in *YUCCA(YUC)* genes, which encode for flavin monooxygenase-like proteins and multiple loss-of-function mutations in *TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS1 (TAA1)* and *TAA1 Related (TAR)* genes (Stepanova et al. 2008; Zhao, 2014) important for auxin synthesis, show embryo phenotypes similar to those observed in auxin signaling mutants, such as *mp* and *bd1* (Cheng et al. 2007).

In summary, formation of an apical-basal body axis seems to form the anchoring basis of all further patterning. The process is very robust and occurs spontaneously in plant cells, unless their polar auxin transport or auxin perception or synthesis is severely compromised by either genetic or pharmacological interference.

Lateral organ initiation in the shoot apical meristem

When the embryo axis forms, two populations of stem cells are set aside at its ends, which will then give rise to all organs of the shoot and root respectively. An important aspect of the patterning of the SAM involves the positioning of lateral organs on its flanks (Okada et al. 1991; Reinhardt et al. 2000; Vernoux et al. 2010; Tobeña-Santamaria et al. 2002; Heisler et al. 2005; Gallavotti et al. 2008; Gallavotti 2013; Traas 2013; Teo et al. 2014). These lateral organs can be leaves of all types, flowers, but also thorns, tendrils or side shoots. Typically, lateral organs are arranged in regular patterns collectively referred to as phyllotaxis. The origin of this pattern has fascinated biologists and mathematicians for centuries (Snow and Snow 1932*a,b*; Steeves and Sussex 1972; Adler et al. 1997; Reinhardt et al. 2000; Kuhlemeier and Reinhardt 2001; Reinhardt et al. 2003; McSteen and Leyser 2005; Jönsson et al. 2006; Reuille et al. 2006; Smith et al. 2006; Kuhlemeier 2007; Stoma et al. 2008; Bartlett and Thompson 2014; Galvan-Ampudia et al. 2016). Mathematical modelling as well as experimental interference studies have shown that the pattern of lateral organs around the SAM, can be explained by assuming that each new lateral organ functions as the source of hypothetical inhibitory influence that subsides over distance and time (Smith et al. 2006). This finding was initially difficult to reconcile with the observation by Reinhardt et al. (2000) that IAA applied locally to the flank of the SAM acts as a positive regulator of phyllotaxis of organ initiation, promoting rather than inhibiting the formation of new lateral organs. The visualization of tentative routes of auxin flow in the SAM (using antibodies against the PIN1 protein) suggested converging

auxin flow in the epidermis, creating a local auxin peak, while simultaneously depleting the tissue of auxin (Figure 3 A). As each peak marks the initiation point for a new primordium, each primordium indeed inhibits the formation of new primordia, but this lateral inhibition mechanism does not involve release of a negative regulator, but instead convergence and depletion of a positive one (Reinhardt et al. 2003). The entire convergence process appears restricted to a single cell layer, the epidermis, tissue that had not been described to be implicated in SAM patterning before. Therefore, it was postulated that in the epidermis unusual auxin transport against a concentration gradient was possible, and several mathematical models were proposed to elaborate this model (Jönsson et al. 2006; Smith et al. 2006). The auxin convergence mechanism is proposed to act parallel to canalization: auxin could converge in concrete spots (up-to-gradient) selectively in the epidermis of the SAM to be then “internalized” (Reinhardt et al. 2003) and transported to subepidermal tissue, where auxin canalization (with-the-flux) would define the position of the vascular strands (Figure 3 B). The interpretation is consistent with the polarities of PIN1 localization in longitudinal sections through the SAM, but live imaging of vascular patterns inside the SAM is precluded by its conical shape (Smith et al. 2006). A clear connection between at least three well characterised auxin transport *PIN* genes and auxin perception at the SAM was recently established with MP acting as a switch by binding the promoter elements in the *PIN* genes (Krogan et al. 2016) and by showing that MP is necessary for localized PIN1 polarity convergence patterns and organ formation (Bhatia et al. 2016).

Lastly, local auxin synthesis, has also been shown to have an impact on proper organ specification at the SAM (Tobena-Santamaria et al. 2002; Cheng et al. 2006, 2007). Moreover, the fact that the loss-of-function mutant phenotypes in the auxin synthesis genes *YUC1* *YUC4* were not rescued by exogenous auxin applications, but could be rescued by expression of the bacterial *iaaM* auxin biosynthesis gene driven by the *YUC1* promoter, led to the conclusion that tissue-specific auxin distribution, and not just absolute auxin supply, is important for patterning processes (Cheng et al. 2006).

The role of auxin in leaf vascular patterning and leaf shape specification

In leaf primordia incipient vascular strands can be visualized much easier than in the SAM. These primordia remain flat and PIN1-GFP fusion proteins can be well visualized in *Arabidopsis* for at

least the first four days of primordium development (Scarpella et al. 2006). During this time, not just the primary vein, but lobes of secondary veins form. In addition, some levels of higher-order venation (3rd, 4th and 5th order veins) are being specified and are visible as PIN1 expression domains. Expression domains of PIN1 in leaf development were not restricted to subepidermal areas presaging future veins, but were also found in the leaf epidermis and the leaf margin, where PIN1 intracellular polarity indicated a reiteration of a point specification process, similar to the one initially specifying the leaf itself. The notion that the cell biological mechanism specifying the position of secondary veins is similar to that specifying the leaf itself and its primary (mid) veins, is also reflected in the developmental analysis of complex leaves. Using complex leaves of the close relative of *Arabidopsis*, *Cardamine hirsuta*, it was shown that early PIN1 dynamics in leaflets of complex leaves were essentially similar to what had been observed in simple *Arabidopsis* leaves, and the initiation of these leaflets along the axis of the rachis had some resemblance to the initiation of the leaves in the SAM (Barkoulas et al. 2008). Thus, for studying the auxin transport dynamics underlying the definition of spots by self-reinforcing auxin accumulation and internalization, the leaf especially a complex leaf, may be more accessible than the SAM itself (Scarpella and Helariutta 2010).

The venation of leaves is highly diverse and certainly influenced by a number of largely unknown genetic cues. However, the adaptive flexibility of venation patterns in many species reveals that there is also a fair amount of self-organizing capacity in the patterning mechanism. Early auxin application experiments (Sachs 1975) and mathematical modeling provided by Mitchison (1980), prompted Sachs and collaborators to propose auxin drainage as a self-organizing principle shaping leaf venation, while alternative models proposed, among other mechanisms, area-subdividing activator-inhibitor interactions as key factors (Meinhardt 1996). The existence of connected veins (i.e. veins connected to other veins on both ends, as opposed to veins ending freely in the leaf lamina) served as one main argument against auxin drainage from dispersed sources in the lamina. This issue was addressed by Scarpella et al. (2006) by showing that at least many connected veins are specified in a polar fashion. When their ontogeny is visualized using the pre-procambial marker AtHB8 (Baima et al. 1995), connected veins are initiated as open-ended branches on pre-existing veins and then extend to build bridges (connected veins), unless all cells in the area are recruited into mesophyll fate and no longer available for procambium formation (Figure 3 C, D). The open-ended origin of closed veins was also visible as gradually extending PIN1 expression domains (Scarpella et

al. 2006). The expression of PIN1 precedes even the AtHB8 marker visibility and also allows for visualization of apparent auxin transport directions in developing veins. As it turned out, future connected veins arise as open-ended with auxin-transport polarity towards pre-existing veins (“basal polarity”). Moreover, connected veins can arise from the merger of two PIN1 expression domains of opposite polarity extending towards each other. Subsequently, such connected PIN1 expression domains consist of two stretches of opposite polarity, connected by a single cell of dual polarity or a bipolar cell (Scarpella et al. 2006, Figure 3 D). The generality of this mechanism in producing connected vein networks also in the higher-order venation system was confirmed by live imaging of leaf development (Wenzel et al. 2007; Sawchuk et al. 2007; Marcos and Berleth 2014). Moreover, besides the intercellular auxin transport mediated by PIN1 it seems that also, intracellular auxin transport mediated by PIN6, PIN8 and PIN5 has a role in the proper patterning of the vascular network (Sawchuk and Scarpella 2013*b*; Sawchuk et al. 2013). Mathematical modeling and experimental refinement have been jointly used to better describe auxin-based vascular tissue patterning processes (Krupinski and Jönsson 2010; Bennett et al. 2014; Lee et al. 2014)

Auxin patterning mechanisms in other plant organs

Auxin has more roles in plant patterning than described in the preceding examples. Pattern acquisition and associated *PIN1* mediated auxin focusing in roots has also been characterized in great detail and has been subjected to mathematical modeling (Swarup et al. 2005; Grieneisen et al. 2007; Mironova et al. 2010; Band and King 2011; Cederholm et al. 2012; Liu et al. 2014; Mellor et al. 2016). In other structures, the roles of auxin are less characterized, in some cases even controversial. Auxin plays a role in shoot branching or apical dominance (McSteen and Leyser 2005; Balla et al. 2011, 2016; Bennett et al. 2014, 2016) and in patterning of the reproductive structures (Dresselhaus and Schneitz 2014; Sehra and Franks 2015). At the floral meristem, local auxin foci mark the outgrowth of the gynoecium and carpel primordia (Larsson et al. 2013, 2014) and the flow of auxin is important for the apical-basal growth of the gynoecium (Zúñiga-Mayo et al. 2014; Marsch-Martínez and de Folter, 2016) although its role in patterning the embryo sac, is still controversial (Pagnussat et al. 2009; Lituiev et al. 2013; Galbiati et al. 2013; Cucinotta et al. 2014). During stomata differentiation auxin functions as a repressor (Balcerowicz and Hoecker 2014; Balcerowicz et al. 2014; Zhang et al. 2014). Although auxin activity is high in young meristemoid stomata cells and important for their asymmetrical division, auxin depletion is necessary for the

transition to the guard mother cell and a switch from the unequal to equal division that gives rise to the two guard cells of a stoma (Le et al. 2014; Livanos et al. 2015, 2016).

It is possible that auxin has been recruited in several otherwise unrelated processes and it is often unclear how exactly auxin controls patterned cell differentiation. Currently, it has not been demonstrated that auxin concentrations are directly translated into cell identities anywhere in the plant, as a classical morphogen concept would demand (Krupinski and Jönsson 2010; Bennett et al. 2014). Alternatively, auxin may just trigger processes in some cells but not others, which would keep the system highly robust with regard to the actual concentrations of auxin (Bennett et al. 2014). Nevertheless, it seems clear that auxin flow is at the core of plant polarity and axis formation and the dynamic changes of auxin flow accompanies the generation of biological patterns in many plant organs.

Conclusions

Over the past two decades, auxin signal transduction through gene regulation and auxin cell-to-cell transport in immature tissue have both become genetically tractable. The identified proteins and their cellular roles and context have opened the door to understand previously identified roles of auxin in vascular patterning processes, as well as to vastly expand this role and to integrate it into programs involving other plant signalling pathways. Conceptually, the most attractive aspect of these discoveries is that they help to understand the amazing self-organizing properties of plant development, whose molecular understanding will dramatically promote plant regeneration strategies in biotechnology and breeding. At least three lines of novel technology and strategy will feed into accelerating progress in understanding plant pattern formation: (a) Functional genomics open the door to integrate the roles of the vast majority of genes, whose functions cannot simply be deduced from loss-of-function mutant phenotypes, understand the cellular mechanisms in greater detail. (b) Novel imaging, including live imaging technologies allows to better trace the roles of multiple relevant proteins in dynamic processes, which will allow to better integrate intra- and intercellular processes. (c) Not least, mathematical modelling will sharpen hypothesis making and testing. In combination with the two other supporting developments, this will gradually enable a systematic understanding of plant patterning events.

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Figure captions

Figure 1. The “up-the-gradient” and “with-the-flux” models of PIN-dependent auxin concentration. (A) In the epidermis cells with low auxin concentrations (pale green), PIN1 is oriented towards the cells with higher auxin concentrations (green) or “up-the-gradient”. When this process is executed by a whole field of cells would give rise to a convergence point (CP). (B) When the cell (CP) accumulates a threshold concentration, PIN1 polarity is thought to shift towards subepidermal tissue to align with the main auxin flux (outlined red cell) or “with-the-flux” which is oriented towards the existing vasculature. This would generate vascular strands in an auxin canalization-like process (Bayer et al. 2009, Bennett et al. 2014).

Figure 2. Schematic representation of *Arabidopsis* embryo development and of the coupled auxin distribution. (A) After the first division of the zygote, PIN7 (blue arrow) promotes the auxin transport from the suspensor (s.) towards the apical cell. (B) Octant stage - the apical-basal subdivision in the upper tier (u.t.) and lower tier (l.t.); auxin is distributed homogenously in the embryo, by PIN1 (red arrows). (C) Dermatogen stage - the protoderm is specified (pd.) through which auxin is distributed through PIN1 towards the apex. (D) Globular stage - the divisions of the inner cells (i.c.) are oriented in the apical-basal direction; auxin flow, directed by PIN1, is internalized towards the uppermost suspensor cell; the flow of auxin by PIN7 in the suspensor is also reverted. (E) Heart stage - the upper tier developed in two symmetrical cotyledon primordia (c.p) and the lower tier to the vascular tissue (v.t); the uppermost suspensor cell forms the hypophyseal cell (h.c.); the auxin flux is oriented in the epidermis (ep.) towards the incipient cotyledons and internalized through the vascular tissue towards the root pole. (F) Mid-torpedo stage the cotyledons (co.) are enlarging as well as the hypocotyl (hy.), the shoot apical meristem (SAM) and the root apical meristems (RAM) are defined (Izhaki and Bowman 2007; Capron et al. 2009; Petrasek and Friml 2009; Wabnik et al. 2013).

Figure 3. Model of auxin flow during leaf and leaf vascular system initiation. (A) In the SAM, auxin transported towards primordia (P) is effectively depleting surrounding epidermis of auxin. At sites least affected by the depletion (i.e. at maximum distance from pre-existing primordia), auxin is transported in all directions through the epidermis towards an auxin maxima (black and white arrows) leading to the formation of a convergence point (CP red) to mark the site of new primordium

initiation. (B) Enlarged view of the box in (A). At the primordia tip, auxin transport is turned towards sub epidermal tissue (internalized) where it continues in the tip to base direction delineating the path of a subsequently forming vascular strand. (C) Leaf lamina with major and minor veins. (D) Expanded view of the box in (C). Auxin is transported through just a single cell file in the epidermis of the leaf margin, to form convergence points associated with the main (1°), second (2°) order veins; the higher order (h) veins are either open with auxin transport oriented towards the major veins or they connect through bipolar cells to form loops (l) (Reinhardt et al. 2003, Scarpella et al. 2006, Berleth et al. 2007).

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45x27mm (600 x 600 DPI)

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93x98mm (600 x 600 DPI)

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93x107mm (300 x 300 DPI)

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