Providing plants with a new sense of direction

Auxin transport

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Plants, like animals, regulate their growth and development using hormone signals. The hormone auxin plays a critical role throughout the plant life cycle, regulating key processes such as embryo patterning, root branching, shoot phyllotaxis and floral development. Auxin is also crucial for regulating plant adaptive responses to environmental signals such as changes in the direction of light, gravity or water (collectively termed tropisms). Auxin therefore functions as a master regulator of plant growth and development (Figure 1). Given this role, determining where and when auxin accumulates in plant tissues is of paramount importance. In this article, we describe how plants control auxin distribution employing a specialized transport mechanism.

Figure 1. Auxin regulates a wide variety of plant processes.

Auxin is unique among plant hormones for exhibiting polar transport (Figure 2). Following its synthesis in the shoot apex and developing leaf primordia, auxin is either mobilized through the phloem or actively transported in a polar manner to its target tissues. To explain the polarity of auxin movement, Rubery and Sheldrake and Raven independently proposed the chemiosmotic hypothesis. Indole-3-acetic acid (IAA), the major form of auxin in higher plants, is a weak acid and at intracellular pH exists in its membrane-impermeant (IAA⁻) form. However, in the acidic extracellular apoplastic pH, IAA will exist in both IAAH (membrane-permeant) form and IAA⁻ forms (Figure 3). Remarkably, two decades before the first auxin transporter was characterized at the molecular level, the chemiosmotic hypothesis not only proposed the existence of specialized auxin carriers, but also hypothesized that the asymmetric localization of auxin transporters provides the basis for the directionality of auxin movement.

Thanks to advances in molecular genetics and cell biology studies in the model plant Arabidopsis thaliana over the last 20 years, we now know that auxin is transported via specialized classes of carrier proteins. Auxin influx carriers facilitate the uptake of auxin into plant cells, whereas auxin efflux carriers mediate the export of auxin from plant cells. In Arabidopsis, the AUXIN1/LIKE-AUX1 (AUX1/LAX) family of auxin transporters represent the major influx carriers, whereas PINFORMED (PIN) and P-GLYCOPROTEIN family members encode the major auxin efflux carriers.

Auxin influx carriers

Auxin influx carriers are encoded by a small gene family comprising four family members: AUX1, LAX1, LAX2 and LAX3 in Arabidopsis (Figures 4a and 4b). These genes encode multi-transmembrane (TM) proteins that are part of a plant specific subclass of the auxin/amino acid permease (AAAP) superfamily. Considering that IAA is structurally similar to tryptophan, this is not surprising from an evolutionary perspective. Mutations in these genes result in auxin-related developmental defects that shed light on their function(s) during plant development. For example, AUX1 regulates root development.
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gravitropism, LAX2 facilitates vascular development and LAX3 controls lateral root emergence, whereas AUX1, LAX1 and LAX2 act redundantly to ensure phyllotactic patterning (i.e. positioning of new leaf primordia) (reviewed by Swarup and Peret). More generally, these studies illustrate that auxin membrane diffusion is clearly limiting, necessitating carrier-mediated uptake.

Auxin efflux carriers

The PIN auxin efflux carrier gene family is found throughout the plant kingdom. The Arabidopsis genome contains eight gene family members, encoding multi-TM proteins that can be subdivided into two subclasses (Figures 4c and 4d). The PIN1 subclass of PIN proteins contain a large central hydrophilic loop and are localized to the plasma membrane. They regulate many aspects of plant growth and development including vascular differentiation (PIN1), root gravitropism (PIN2 and PIN3), patterning of the root stem cell niche (PIN4) and embryo (PIN7). The other PIN subclass contains three members, PIN5, PIN6 and PIN8, and are localized to the endoplasmic reticulum (ER). These members either lack the central hydrophilic loop (PIN5 and PIN8) or this loop is reduced (PIN6). The PIN5 subclass of PIN proteins appears to play a key role in intracellular auxin movement and auxin homoeostasis. The importance of intracellular auxin transport is highlighted further by the discovery of another PIN-LIKE (PILS) gene family. Two members of this novel family, PILS2 and PILS5, are also localized to the ER. These ER-localized PIN and PILS proteins appear crucial for hormone homoeostasis by sequestering this signal away from the nuclear-localized auxin receptor.

Besides PIN auxin efflux carriers, two members of the P-GLYCOPROTEIN (PGP) class of ABC (ATP-binding cassette) transporters, ABCB1 and ABCB19, have been reported to regulate auxin efflux. ABC transporters are integral membrane proteins that regulate the efflux of a wide range of substrates using energy from ATP hydrolysis (Figures 4e and 4f). The Arabidopsis ABC transporter family is a big gene family, but only three members of the family have been shown to regulate auxin transport. Although the evidence for ABCB1 and ABCB19 as auxin efflux carriers is well established, the proposed auxin influx activity of another family member, termed ABCB4, is more controversial (reviewed by Swarup and Peret).

Figure 2. Auxin transport is polar in nature. (a) Cartoon showing polarity of auxin movement. (b) Polarity of auxin movement (arrows) is mainly determined by asymmetric localization of PIN auxin efflux carriers. Confocal images showing PIN1 and PIN2 (green) localization in Arabidopsis root apex. Insets: magnified views to show polar pin localization (arrowheads).

Figure 3. The chemiosmotic auxin transport model predicts that IAA transport is predominantly carrier-mediated. IAA is a weak acid and at cellular pH exists in its membrane-impermeant IAA\(^{-}\) form and requires auxin efflux carriers to transport it across the membrane. At apoplastic pH (5.5), the majority of IAA is present in its dissociated IAA\(^{-}\) form and requires active (carrier-mediated) uptake, whereas a small fraction of the protonated form (IAAH) can passively diffuse across the membrane.
Another class of plasma-membrane-localized transporter has recently been reported to facilitate auxin transport. NRT1.1 normally functions as a transreceptor involved in perception/transduction of nitrate, a key nutrient and signal in plants. However, heterologous expression studies have provided evidence that NRT1.1 can also transport auxin. Consistent with such a role, studies in Arabidopsis suggest that nitrate and auxin compete with each other to be taken up by NRT1.1 into lateral root cells, resulting in nitrate-suppressing organ emergence. This provides a very attractive mechanism for the developmental effects of auxin to be linked to nutrient status in the soil.

**Figure 4.** Three major families of auxin transporters in Arabidopsis. Phylogenetic trees of the AUX/LAX (a), PIN (c) and PGP (e) protein sequences generated from a Clustal Omega alignment. Typical organizations of transmembrane domains in AUX/LAX (b), PIN (d) and ABC transporters (f). The AtPGP transporter family is a very big gene family and only members with roles in auxin transport are shown for simplicity.

**NRT1.1**

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### Polarity of auxin movement and plant development

It is now well established that the polarity of auxin movement is primarily determined by the asymmetric subcellular localization of PIN proteins. For example, PIN1 is localized on the basal (termed rootward) face of root vascular cells facilitating auxin movement towards the root apex. In contrast, PIN2 is asymmetrically localized at the apical (shootward) face of epidermal cells and basal (rootward) face of cortical cells. PIN localization and polarity is post-translationally regulated by phosphorylation of the large central hydrophilic loop (Figure 4d).

The pattern of PIN subcellular localization can be dynamic and is often closely linked to environmental signals. In roots, a gravitropic stimulus is perceived in specialized gravity-sensing columella cells at the root tip, but root bending takes place in the elongation zone (Figure 5). Auxin transporters facilitate movement of auxin from the tissue sites of gravity perception to gravitropic response. PIN3 and PIN7 are initially localized on every face of gravity-sensing columella cells, but relocalize to the bottom face of these cells in response to a gravity stimulus. This provides an elegant mechanism to establish a lateral auxin gradient by diverting hormone transport to just one side of the root. We recently visualized the gravity-induced lateral auxin gradient (Figure 5) using negatively and positively regulated auxin reporters DII–VENUS and DR5–VENUS respectively. Both AUX1 and PIN2 then channel the lateral auxin gradient via the lateral root cap to cells in the elongation zone. Auxin transport in elongation zone tissues is facilitated by AUX1 and PIN2 as well as ABCB1 and ABCB19. Computer simulations of auxin fluxes through elongation zone tissues suggest that the combined activities of AUX1 and PIN2 in the expanding epidermal cells minimizes the effect of radial diffusion while facilitating shootward auxin transport and maintaining a differential auxin gradient. Besides, regulation of PIN2 levels between upper and lower sides of the root help create a much sharper auxin gradients. Differential accumulation of auxin between the upper and lower sides of a gravity-stimulated root results in differential growth that causes root bending (Figure 5). Hence auxin transport is critical for providing plants with a sense of direction.

Differential expression and localization of auxin transporters (notably PIN and AUX1/LAX proteins) in a tissue-specific manner result in the creation of auxin gradients, providing developmental cues that appears to be a prerequisite for organ development. The directionality of PIN-mediated transport serves as a polarity determinant during embryo patterning. Changes in PIN1 and PIN7 polarity during embryogenesis cause redirection of auxin...
fluxes and facilitate creation of auxin gradient and specification of presumptive embryo root pole\(^1\). Mutations such as \textit{gnom} affect polar PIN localization during embryogenesis, resulting in loss of embryo polarity and an embryo/seedling lethal phenotype\(^2\).

As in embryogenesis, auxin distribution patterns lateral root morphogenesis. Auxin is important not only for lateral root initiation, but also for lateral root emergence. Lateral roots originate from pericycle cells deep inside the primary root, necessitating that they penetrate through several overlying layers of cells. The auxin influx carrier LAX3 has been shown to facilitate lateral root emergence\(^1\). Auxin originating from the lateral root primordia induces LAX3 expression in the cortical and epidermal cells directly next to the lateral root primordia. This creates an auxin sink in several cells in front of the primordia that leads to induction of cell-wall-remodelling enzymes that facilitates organ emergence. These examples highlight the importance of auxin transport and its carrier proteins to create auxin gradients in plant tissues.

**References**


Malcolm Bennett has pioneered the genetic analysis of root growth and development over the last 20 years, identifying a number of key root regulatory genes and signals including AUX1, the first auxin transport protein to be described in plants. Over the last decade he has pioneered systems approaches in plants, helping to establish the BBSRC/ EPSRC Centre for Plant Integrative Biology (CPIB) and currently serving as its Director. BBSRC Professorial Fellowship, ERC Advanced Investigator and Wolfson-Royal Society awards have recently enabled him to assemble a multidisciplinary research team to image roots growing in soil. email: Malcolm.Bennett@nottingham.ac.uk

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**Figure 5.** Root gravitropism. (a) Auxin influx and efflux carriers facilitate auxin movement following a gravity stimulus. (b–d). Confocal image showing PIN2 (green), PIN3 (green) and AUX1 (yellow) localization in root apex. (d–f). Upon gravity perception, asymmetric localization of PIN3 in the columella cells results in differential auxin movement between upper and lower side of a gravity-stimulated root as seen by asymmetric localization of the auxin-response reporters DII–VENUS (d) and DR5–VENUS (e). An interpretation of differential auxin movement inferred from (d) and (e) is shown in (f). Gravity vector (g) is indicated with an arrow. Adapted from Swarup et al. (2013) In Roots: the Hidden Half, 4th edn (Eshel, A. and Beeckman, T., eds), pp. 19-1–19-34, CRC Press, Boca Raton.