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Seven Things We Think We Know about Auxin Transport

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ABSTRACT Polar transport of the phytohormone auxin and the establishment of localized auxin maxima regulate embryonic development, stem cell maintenance, root and shoot architecture, and tropic growth responses. The past decade has been marked by dramatic progress in efforts to elucidate the complex mechanisms by which auxin transport regulates plant growth. As the understanding of auxin transport regulation has been increasingly elaborated, it has become clear that this process is involved in almost all plant growth and environmental responses in some way. However, we still lack information about some basic aspects of this fundamental regulatory mechanism. In this review, we present what we know (or what we think we know) and what we do not know about seven auxin-regulated processes. We discuss the role of auxin transport in gravitropism in primary and lateral roots, phototropism, shoot branching, leaf expansion, and venation. We also discuss the auxin reflux/fountain model at the root tip, flavonoid modulation of auxin transport processes, and outstanding aspects of post-translational regulation of auxin transporters. This discussion is not meant to be exhaustive, but highlights areas in which generally held assumptions require more substantive validation.

Key words: Auxin transport; ABCB; AUX1; PIN; phototropism; gravitropism; fountain model; shoot branching; leaf expansion; venation; flavonoids.

INTRODUCTION

Polar streams of the phytohormone auxin regulate organ development, elongation, shoot/root branching, and plastic growth responses to light, gravity, and touch in plants (reviewed in Zazímalová et al., 2010). Auxin is synthesized mainly in the shoot apex and young leaves (Ljung et al., 2001, 2005), and is transported in polarized streams via auxin transporters. This directional transport is coordinated by AUXIN RESISTANT1/LIKE AUX1 (AUX1/LAX) uptake permeases (TC 2.A.18), ATP Binding Cassette subfamily B (ABCB) transporters (TC 3.A.1), and PIN-FORMED (PIN) carrier proteins (TC 2.A.69) and is motivated by chemiosmotic gradients (Rubery and Sheldrake, 1974; Raven, 1975; Li et al., 2005; reviewed in Benjamins and Scheres, 2008; Petrasek and Friml, 2009; Zazímalová et al., 2010).

At apoplastic pH (~5.5), the principal auxin, indole-3-acetic acid (IAA, pKa = 4.75), enters cells via lipophilic diffusion and anionic uptake mediated by the proton symporters of the AUX1/LAX family (Bennett et al., 1996; Swarup et al., 2005; Yang et al., 2006; Yang and Murphy, 2009). ABCB4 appears to contribute to uptake in root epidermal cells when auxin levels are low (Santelia et al., 2005; Terasaka et al., 2005; Yang and Murphy, 2009) and a dual-function auxin/nitrate transporter, NRT1.1, contributes to auxin uptake in roots under nitrogen deficiency conditions (Krouk et al., 2010). At cytosolic neutral pH, IAA is anionic and requires facilitators or transporters to exit cells. Full-length PIN carriers mediate tissue-specific directional cellular auxin export and a subset of ABCB transporters function coordinately with PIN proteins in exclusion/export of auxin from the plasma membrane (Titapiwatanakun et al., 2008; reviewed in Zazímalová et al., 2010).

The map of transporter activities becomes more complex with every publication, especially as information derived from studies in *Arabidopsis* is extended to other species. However, a basic overview would include the following: PIN1 and ABCB19 are the primary mediators of rootward auxin transport in the stele (Okada et al., 1991; Noh et al., 2001) while AUX1, PIN2, and ABCB4 mediate shootward auxin flows from the root apex (Luschnig et al., 1998; Marchant et al., 2002; Terasaka et al., 2005; Lewis et al., 2007). PIN1 is the primary mediator of polar auxin flows during embryo- and organogenesis with PIN7 contributing to maintenance of auxin supply to

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meristems (Friml et al., 2003). Auxin movement out of the shoot meristem is dependent on PIN1, PIN7, ABCB1, ABCB19, and AUX1 (Steinmann et al., 1999; Friml et al., 2003; Reinhardt et al., 2003; Geisler et al., 2005; Blakeslee et al., 2007), and mobilization of auxin out of the root meristem is dependent on PIN3, PIN4, AUX1, ABCB1, and, to a lesser extent, ABCB19 (Geisler et al., 2005; Bouchard et al., 2006; Blakeslee et al., 2007). Lateral root formation is dependent on rootward vascular auxin flows, but involves transport activity of ABCB1 and epidermal auxin uptake by LAX3 during emergence (Geisler et al., 2005; Swarup et al., 2008). Other transport processes are dependent on environmental conditions. For example, PIN1, PIN3, ABCB19, and AUX1/LAX proteins appear to function in phototropic responses (Noh et al., 2001; Friml et al., 2002b; Nagashima et al., 2008; Stone et al., 2008; Keuskamp et al., 2010), ABCB4 functions in regulation of root hair elongation (Santelia et al., 2005; Cho et al., 2007), and NRT1.1, which is localized to root stele cells as well as the developing root cap and epidermal cells of post-induction lateral roots, mediates auxin uptake under nitrogen deficiency (Krouk et al., 2010).

AUX1/LAX Auxin Influx Carriers

The auxin influx carrier family comprises four members in *Arabidopsis*: AUX1 and LAX1, 2 and 3 (Parry et al., 2001). AUX1/LAX transporters belong to the amino acid permease family of plasma membrane (PM) H^+-symporters (reviewed by Kerr and Bennett, 2007). High-affinity auxin import activities of AUX1 and its most similar ortholog, LAX3, have been demonstrated in heterologous systems (Yang et al., 2006; Swarup et al., 2008; Yang and Murphy, 2009). Lower-affinity uptake has also been shown for LAX1 and 2, which exhibit decreased sequence similarity to LAX3 (Parry et al., 2001; Swarup et al., 2008). AUX1/LAX proteins are mobilized to and from the PM by dynamic processes (Grebe et al., 2002; Kleine-Vehn et al., 2006). Mathematical models indicate that AUX1/LAX uptake activities create sinks (Kramer, 2004; Swarup et al., 2005; Kramer and Bennett, 2006) that load and redirect auxin fluxes regulating embryonic root, lateral root, leaf, and apical hook development and motivate the shootward auxin stream that is differentially redirected in root gravitropisim (Marchant et al., 2002; Swarup et al., 2005; Bainbridge et al., 2008; Swarup et al., 2008; Ugartechea-Chirino et al., 2010; Vandenbussche et al., 2010). The contributions of AUX1/LAX proteins in generating auxin sinks are shown by the total loss of gravitropic responses in the *aux1* mutant and the inhibition of lateral root emergence when LAX3 is missing from cortical and epidermal cells that overlay lateral root primodia (Mirza et al., 1984; Bennett et al., 1996; Swarup et al., 2008). Additionally, AUX1 is also localized in the shoot, and therefore plays a role in rootward auxin transport in the shoot (Marchant et al., 2002; Vandenbussche et al., 2010) and possibly in the root under some conditions (Negi et al., 2008). Therefore, we know that AUX1/LAX proteins are essential to gravitropism in primary roots and lateral root emergence, but how they function in these processes and regulatory interactions is still unknown.

PIN Efflux Carriers

The PIN auxin efflux carriers are distantly related to some fungal transporters with 9–11 transmembrane helices, but are thought to have differentiated into a discrete group early in vascular plant evolution (Galvan-Ampudia and Offringa, 2007; Peer and Murphy, 2007; Krecek et al., 2009). The PIN nomenclature is derived from the *PINFORMED* inflorescence phenotype associated with loss of PIN1, which is the primary mediator of polar auxin flow functioning in angiosperm development. The *Arabidopsis* genome contains eight PIN genes, five of which encode full-length PINs (PIN1, 2, 3, 4, and 7) and three of them encode short PINs (PIN5, 6, and 8). Short PIN proteins lack the long central hydrophilic loop found in full-length PINs and are localized to endomembrane structures where they are thought to function in homeostatic auxin compartmentalization (Mravec et al., 2009), although the motive force maintaining the endomembrane auxin gradient has yet to be defined. Auxin efflux directly mediated by PIN1, 2, 4, 5, and 7 has been demonstrated in multiple heterologous systems (Chen et al., 1998; Geisler et al., 2005; Petrášek et al., 2006; Blakeslee et al., 2007; Yang and Murphy, 2009; Kim et al., 2010).

PIN1 and 2 exhibit primarily polar localizations on the plasma membrane while PIN3, 4, and 7 exhibit both polar and apolar plasma membrane localizations, depending on the cell type and external stimuli (Friml et al., 2002a, 2002b, 2003; Wisniewska et al., 2006; Blilou et al., 2005). PIN1, 4, and 7 mediate the polar auxin fluxes that are required to maintain fundamental processes of development and organogenesis during all aspects of plant growth (reviewed in Zazímalová et al., 2007). PIN2 functions in auxin reflux in root tip and root gravitropism (Chen et al., 1998; Müller et al., 1998; Friml et al., 2004; Rahman et al., 2010). PIN3-functions in restriction of auxin in auxin streams and redirection of auxin for directional growth (Friml et al., 2002b). All full-length PINs are trafficked by dynamic cellular mechanisms to a greater or lesser extent, and these processes determine their polar localization (reviewed in Grunewald and Friml, 2010). Therefore, we know that various PINs function in embryo- and organogenesis, gravitropism, and maintenance of the root meristem, but the role of PINs in phototropism and branching is unclear, and how they function in these processes and regulatory interactions is still unknown.

ABCB/PGP ATP-Dependent Auxin Transporters

The ABCB/PGP P-glycoproteins utilize energy derived from ATP hydrolysis to transport amphipathic and anionic molecules (reviwed by Geisler and Murphy, 2006; Peer and Murphy, 2007; and Verrier et al., 2008). To date, the auxin transport activity of *Arabidopsis*, maize, and sorghum ABCB1 and *Arabidopsis* ABCB4 and ABCB19 have been demonstrated *in planta* and in heterologous systems (Noh et al., 2001; Multani et al., 2003; Geisler and Murphy, 2006; Petrášek et al., 2006; Blakeslee et al., 2007; Cho et al., 2007; Lewis et al., 2007;