Chapter 4
Intracellular Auxin Transport

David Scheuring and Jürgen Kleine-Vehn

Abstract The phytohormone auxin is of fundamental importance in plant development. Since the identification of auxin as a plant growth substance, auxin transport has drawn considerable research attention. Intercellular (polar) auxin transport contributes to its graded distribution in cell files or entire organs and allows for dynamic, environmentally controlled rearrangements in auxin accumulation. Insights into polar auxin transport mechanisms have broadened our understanding of the phenotypic flexibility of plants. Besides intercellular auxin transport, auxin is also transported intracellularly across organelle membranes, but its importance in plant development remains nascent. The intracellular sequestration of auxin into cellular compartments, such as peroxisomes and the endoplasmic reticulum (ER), plays important roles in auxin metabolism and could furthermore, in the case of the ER, have a direct impact on auxin signaling events. In this chapter, we review the most recent insights into intracellular auxin transport and its role in cellular auxin homeostasis.

1 Introduction

The phytohormone auxin is an important regulator of plant growth and patterning (reviewed in Leyser 2011). Auxin controls diverse developmental aspects, such as apical–basal axis formation during embryogenesis, de novo postembryonic organogenesis, or pathogen interaction (reviewed in De Smet and Jurgens 2007; Péret et al. 2009; Kazan and Manners 2009). At a cellular level, auxin determines the rate of cell division, elongation, and differentiation (reviewed in Perrot-Rechenmann

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E. Zažímalová et al. (eds.), *Auxin and Its Role in Plant Development*,
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2010). Given its manifold functions, it is not too surprising that auxin levels and the cellular responsiveness to auxin are tightly regulated. It now appears that auxin metabolism and transport jointly control the spatiotemporal activity of auxin (reviewed in Rosquete et al. 2012), which is a key component in regulating plant development.

Four distinct endogenous auxin molecules, namely, indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), 4-chloroindole-3-acetic acid (4-CI-IAA), and phenylacetic acid (PAA), have been identified (reviewed in Simon and Petrášek 2011), from which IAA appears to be the most abundant (reviewed in Strader and Bartel 2011). IAA is mainly synthesized in the young aerial parts of the plant (Ljung et al. 2001) and is subsequently transported in a root-ward direction (reviewed in Petrášek and Friml 2009). Moreover, local auxin biosynthesis throughout the entire plant has been shown to be developmentally important (Zhao 2012; Ljung 2013). The passage of auxin from cell to cell is carried out by multiple auxin carriers at the plasma membrane. The most prominent are the influx carrier AUXIN RESISTANT 1 (AUX1), a subfamily of ATP-BINDING CASSETTE (ABC) transporters, and the PIN-FORMED (PIN) efflux carrier family (reviewed in Grunewald and Friml 2010; Kramer and Bennett 2006; Zažímalová et al. 2010) (Fig. 4.1). Carrier-dependent polar auxin transport contributes to the enormously flexible regulation of auxin distribution within tissues (see Chap. 5).

Intracellular auxin must be perceived and converted into a signal to induce specific cellular auxin responses (see Chap. 6). In *Arabidopsis*, three independent auxin receptors have been proposed (Fig. 4.1): (1) the F-box protein TRANSPORT INHIBITOR RESISTANT1/AUXIN SIGNALING F-BOX (TIR1/AFB) (Dharmasiri et al. 2005; Kepinski and Leyser 2005), (2) the F-box protein S-PHASE KINASE-ASSOCIATED PROTEIN 2A (SKP2A) (Jurado et al. 2010), and (3) the AUXIN-BINDING PROTEIN (ABP1) (Hertel et al. 1972; Tromas et al. 2010).

Most auxin responses have been associated with nuclear TIR1/AFB action (Tan et al. 2007; Dharmasiri et al. 2005). In the absence of auxin, AUXIN/INDOLE ACETIC ACID (Aux/IAA) proteins bind and inhibit AUXIN RESPONSE FACTOR (ARF) transcription factors. Auxin binding to the co-receptor complex of TIR1/AFB and Aux/IAA induces ubiquitination and degradation of Aux/IAA and ultimately the release of ARFs, leading to transcriptional reprogramming (reviewed in Chapman and Estelle 2009).

Nuclear SKP2A is involved in cell cycle regulation and furthermore has been shown to directly bind auxin (Jurado et al. 2008; del Pozo et al. 2006). Mutations in the proposed auxin-binding pocket abolish auxin binding and SKP2A activity (Jurado et al. 2010). Further insight is needed to link SKP2A action and auxin involvement in the cell cycle.

ABP1 was the first auxin-binding protein described in the literature and has a long-standing history as a putative auxin receptor (reviewed in Sauer and Kleine-Vehn 2011; Shi and Yang 2011; Scherer 2011). ABP1 localizes mainly to the ER, but a small portion is secreted to the cell wall where it is assumed to be active (Sauer and Kleine-Vehn 2011). ABP1 responds rapidly and non-genomically to

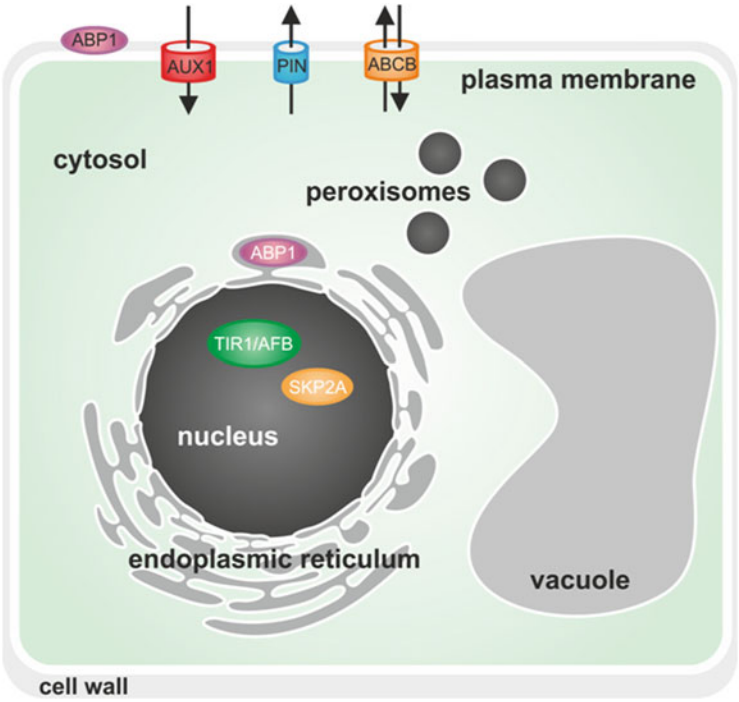

Fig. 4.1 Intercellular auxin carriers and auxin receptors in plants. In *Arabidopsis*, three auxin receptors and at least three different types of auxin carriers at the plasma membrane have been suggested. *Abbreviations*: ABP1 = AUXIN-BINDING PROTEIN1, AUX1 = AUXIN RESISTANT1, PIN = PIN-FORMED, ABCB = ATP-BINDING CASETTE B, TIR1/AFB = TRANSPORT INHIBITOR RESISTANT1/AUXIN SIGNALING F-BOX, and SKP2A = S-PHASE KINASE-ASSOCIATED PROTEIN 2A

auxin by regulating endocytosis or organizing the cytoskeleton (Robert et al. 2010; Xu et al. 2010). These functions occur proximate to the plasma membrane; ABP1 additionally affects the cell cycle and gene expression (David et al. 2007; Braun et al. 2008; Paque et al. 2014).

Both intracellular and extracellular auxin perception is required for integrative auxin signaling. Carrier activity at the plasma membrane might therefore play a decisive role in regulating the balance between extra- and intracellular signaling. Compared with auxin perception and signaling events, auxin metabolism appears at least in part compartmentalized. The precise subcellular distribution of auxinic compounds requires specific auxin transport across organelle membranes, which adds further complexity to the mechanisms of auxin perception and metabolism. However, in contrast with cell-to-cell auxin transport, intracellular auxin transport is only poorly understood. Here, we review our current knowledge of intracellular auxin compartmentalization and transport.

2 Intracellular Auxin Transport at Peroxisomes

IBA, long considered as synthetic auxin, has been detected in plants (reviewed in Ludwig-Müller et al. 1993; Korasick et al. 2013), although a recent study did not detect endogenous IBA in *Arabidopsis* (Novák et al. 2012). IBA, which differs from IAA only in the length of its side chain, is produced by IBA synthase using IAA as substrate. However, other precursors have also been described (reviewed in Woodward and Bartel 2005). IBA displays only weak signaling activity, if at all, and it has been suggested that it functions as a storage form of IAA (reviewed in Korasick et al. 2013). IBA and IAA are both transported from cell to cell, but the underlying carrier systems are distinct (Rashotte et al. 2003). Members of the ABCG transporter family have been propounded as IBA transporters, specifically at the root–soil interface (Růžčcka et al. 2010; Strader and Bartel 2011); however, the biological significance of resulting IBA release into the rhizosphere remains so far unknown. Intercellular IBA carriers still need to be unearthed, but it appears that IBA transport and local conversion to IAA are important during lateral root development (De Rybel et al. 2012). Hence, spatiotemporal conversion of IBA to IAA provides an elegant mechanism to fine-tune auxin biology.

The conversion of IBA to IAA is reminiscent of fatty acid ß-oxidation and most likely involves the same machinery. Similar to fatty acid ß-oxidation, IBA to IAA conversion takes place in peroxisomes (Fig. 4.2) and, hence, also requires IBA and IAA to be imported into and exported from this organelle (reviewed in Hu et al. 2012; Fawcett et al. 1960).

The ATPase PXA1/ABCD1, initially described as transporting fatty acids at peroxisomes (reviewed in Linka and Weber 2010), might be involved in pumping IBA from the cytosol into the peroxisome (Fig. 4.2). Loss-of-function mutants of PXA1/ABCD1 are IBA resistant (Zolman et al. 2001) and show reduced IBA to IAA conversion (Strader et al. 2010). Notably, defects of *abcd1* mutants, such as a reduced number of lateral roots (Zolman et al. 2001) and a delayed stamen filament elongation (Footitt et al. 2007), could be partially rescued by exogenous auxin application.

These findings suggest that IBA transport into peroxisomes and subsequent conversion to IAA is of importance for plant growth and development. However, the identity of transporters mediating the export of IBA-derived IAA from peroxisomes into the cytosol remains unfortunately elusive (Fig. 4.2).

3 Compartmentalization of Auxin in the Endoplasmic Reticulum

The endoplasmic reticulum has a prominent role in the synthesis and folding of proteins and is also involved in multiple metabolism pathways. Recent evidence suggests that the ER is essential for auxin metabolism (reviewed in Barbez and

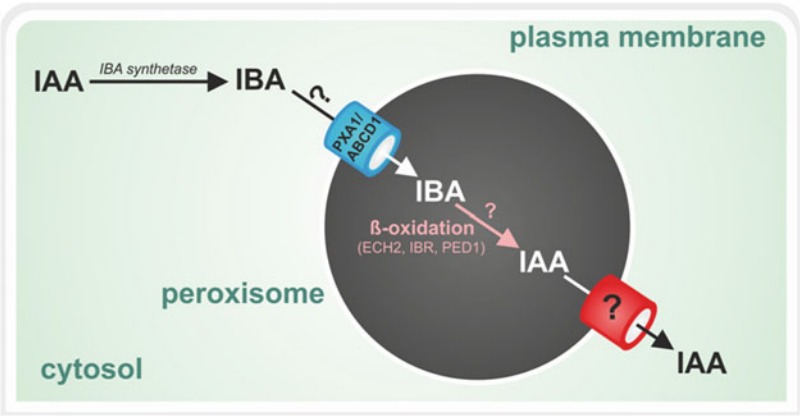

Fig. 4.2 Auxin transport at peroxisomes. Conversion from IAA to IBA is carried out in the cytosol by the IBA synthetase. The reverse reaction, IBA to IAA takes place in peroxisomes, but the exact mechanism remains unclear. However, enzymes acting in the ß-oxidation pathway have been suggested as playing an important role in IBA to IAA conversion. ABCD1 is likely to transport IBA into peroxisomes. The export carrier for IAA is still unknown. *Abbreviations*: IAA = Indole-3-acetic acid, IBA = Indole-3-butyric, and PXA1/ABCD1 = ABC transporter. ECH2, IBR, and PED1 are candidates for IBA ß-oxidation

Kleine-Vehn 2013). For example, several auxin amid-conjugate hydrolases show an ER-retention motif (Campanella et al. 2003; Bitto et al. 2009), suggesting that conjugation-based regulation of auxin activity might be linked to the ER. A splice variant of the IAA biosynthesis gene YUCCA4 also localizes to the cytosolic face of the ER (Kriechbaumer et al. 2012). The functional importance of this localization is currently unknown, but could supply auxin for ER-directed transport. Furthermore, the auxin receptor ABP1 is localized within the ER lumen and, hence, auxin signaling could also take place in the ER (Barbez and Kleine-Vehn 2013).

While all these findings indicate that the ER might be important for auxin metabolism and signaling, it is also clear that such compartmentalized mechanisms would require auxins or auxinic compounds to be transported across the ER membrane. Therefore, it was not too surprising that putative auxin transporters were indeed discovered at the ER. However, the actual molecular player was unexpected, because the transporter family has been already identified as being involved in intercellular auxin transport.

3.1 PIN Subfamily of Auxin Carriers Function at the ER

PIN proteins are auxin efflux carriers (see Chap. 5), originally said to function in intercellular auxin transport, modulating an extensive range of developmental

processes (reviewed in Grunewald and Friml 2010). In *Arabidopsis thaliana*, all 8 members of the PIN protein family share two regions of multiple transmembrane domains, symmetrically separated by a hydrophilic loop. PIN1-4 and PIN7 have long hydrophilic loops and localize to the plasma membrane, where they facilitate auxin efflux. This subgroup of PIN proteins determines the rate and direction of intercellular (polar) auxin transport (Wisniewska et al. 2006; Petrášek et al. 2006). On the contrary PIN5, PIN6, and PIN8 display a shorter hydrophilic loop and localize to the ER (Mravec et al. 2009; Dal Bosco et al. 2012; Ding et al. 2012; Sawchuk et al. 2013). Thus, the classification of PINs according to the length of their central hydrophilic loop correlates with their distinct intracellular localization (Mravec et al. 2009). The sequence requirements for an ER exit may lie in the loop region (Ganguly et al. 2014).

It is intriguing that PIN auxin carriers appear to regulate both intercellular and intracellular auxin transport and that ER-localized PINs seemingly predate the evolution of plasma membrane localized PINs (Viaene et al. 2013). ER-localized PIN5 increases cellular auxin retention, presumably by transporting auxin from the cytosol into the ER lumen (Mravec et al. 2009). The availability of cytosolic/nuclear IAA would be reduced as a result of such auxin compartmentalization in the ER, which in turn might impact on nuclear auxin signaling events (Mravec et al. 2009).

Apart from the transport of free IAA, PIN activity at the ER has been linked to auxin conjugation-based metabolism (Mravec et al. 2009; Dal Bosco et al. 2012; Ding et al. 2012). Only a small fraction of auxin is in its free and active form—the majority is conjugated to amino acids, peptides, and sugars (see Chaps. 2 and 3). Conjugation enables cells to reversibly inactivate and store auxin. In *Arabidopsis*, IAA is mainly conjugated to amino acids and the most abundant IAA amid conjugates are IA-alanine (Ala), IA-leucine (Leu), IA-aspartate (Asp), and IA-glutamate (Glu) (Tam et al. 2000; Kowalczyk and Sandberg 2001). The auxin conjugation to amino acids is catalyzed by members of the auxin-inducible GRETCHEN HAGEN3 (GH3) gene family (Staswick et al. 2005; Hagen and Guilfoyle 1985), while hydrolysis of amino acids is achieved by the IAA-LEUCINE RESISTANT 1 (ILR1)-like family (Bartel and Fink 1995; Rampey et al. 2004; Davies et al. 1999; LeClere et al. 2002). PIN5 activity correlates with higher levels of IAA amino acid and sugar conjugates. Hence, it has been hypothesized that PIN5 leads to auxin accumulation in the ER, where conjugation-based metabolism might take place (Mravec et al. 2009) (Fig. 4.3). Similar to PIN5, PIN8 localizes to the ER (Dal Bosco et al. 2012; Ding et al. 2012). Ectopic PIN8 expression leads to aberrant hypocotyl and root length as well as changes in flowering time (Ding et al. 2012; Dal Bosco et al. 2012). Surprisingly, a *pin5* loss-of-function mutation can rescue the *pin8* defects in pollen, whereas *PIN5* overexpression attenuates phenotypes caused by ectopic PIN8 expression (Ding et al. 2012). These findings indicate antagonistic roles for PIN5 and PIN8, possibly by counteracting the sequestration of auxin and auxin conjugates in the ER lumen (Ding et al. 2012). Similar to the scenario in pollen, *pin6* and *pin8* show antagonistic genetic interaction with *pin5* in the control of leaf vein patterning (Sawchuk

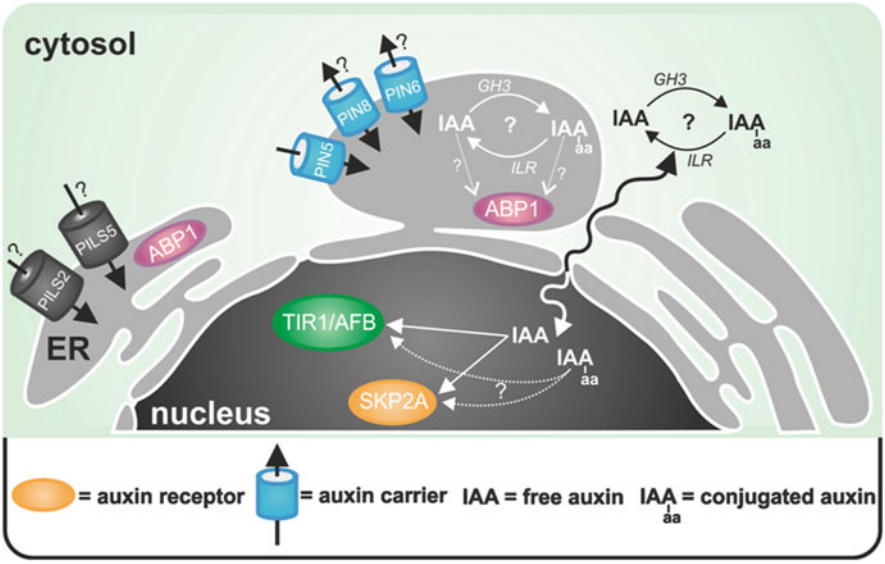

Fig. 4.3 Simplified model of auxin transport at the ER. Distinct auxin pools in the cytosol, the nucleus, and the ER depend on different carriers (as depicted in the *cartoon*). *Abbreviations*: ER = endoplasmic reticulum, IAA = Indole-3-acetic acid, PIN = PIN-FORMED, PILS = PIN-LIKES, ABP1 = AUXIN-BINDING PROTEIN1, GH3 = GRETCHEN HAGEN 3, and ILR = IAA-LEUCINE RESISTANT1-like

et al. 2013) (Fig. 4.3). This could be explained by a scenario in which PIN5 transports auxin from the cytoplasm into the ER (Mravec et al. 2009), and PIN6/PIN8 transport auxin from the ER to the cytoplasm (Sawchuk et al. 2013), but may also depend on PIN protein translocation to the plasma membrane (Ganguly et al. 2014). Notably, intercellular (PIN1) and intracellular (PIN5, PIN6, and PIN8) auxin transport seem to converge to fine-tune auxin-dependent vein networks (Sawchuk et al. 2013), suggesting complex interplay between intercellular and ER auxin transport.

3.2 *PILS Proteins: A New Family of Putative Auxin Transporters*

Besides PIN proteins, another family of putative auxin transporters at the ER has been identified, possibly highlighting the importance of auxin compartmentalization in the ER (Barbez et al. 2012). PIN-LIKES (PILS) proteins display only low sequence similarity and seem evolutionarily distinct from PINs (Feraru et al. 2012; Viaene et al. 2013), but show similarities to PINs in the predicted protein topology (Barbez et al. 2012). PILS proteins are conserved throughout plant evolution (Barbez et al. 2012; Feraru et al. 2012), whereas PIN proteins are absent in

unicellular algae (Viaene et al. 2013). PILS2 and PILS5 gain- and loss-of-function mutants show auxin-related phenotypes and convey resistance and hypersensitivity to exogenous auxin application, respectively (Barbez et al. 2012). PILS2 and PILS5 increase cellular auxin accumulation at the ER (in plant and heterologous systems), reduce auxin signaling, and stimulate auxin conjugation (Barbez et al. 2012; Barbez and Kleine-Vehn 2013). This analogy proposes a PIN5-like function for PILS2 and PILS5 in auxin transport from the cytosol into the ER lumen (Mravec et al. 2009; Barbez et al. 2012). However, possible functional PILS/PIN redundancy or distinct modes of actions remain to be addressed. Recently, a connection between ER-based auxin homeostasis and the unfolded protein response (UPR) has been proposed. Upon ER stress, auxin receptors and transporters are transcriptionally downregulated in *Arabidopsis* seedlings (Chen et al. 2013). Remarkably, *pin5* as well as *pils2 pils5* mutants show a reduced UPR activation, suggesting a carrier-dependent link between auxin compartmentalization in the ER and UPR (Chen et al. 2013).

4 Auxin and Other Cellular Compartments

Auxin metabolism and/or compartmentalization in peroxisomes and ER lumen is currently gaining research attention, raising questions about the involvement of additional organelles contributing to auxin homeostasis. Recently, the vacuole and mitochondria have been advanced as playing a role in cellular auxin homeostasis.

The *Arabidopsis* transporter protein WALLS ARE THIN1 (WAT1) is ubiquitously expressed throughout the plant, but it is preferentially associated with vascular tissues. Within the cell, WAT1-GFP localizes to the tonoplast (Ranocha et al. 2010). In *wat1-1* mutants, the cell wall is defective (Denance et al. 2012), but local auxin application rescues this mutant phenotype (Ranocha et al. 2013). Auxin transport assays, using isolated *Arabidopsis* vacuoles and heterologous systems, such as yeast and *Xenopus* oocytes, revealed that WAT1 is transporting auxin, indicating that WAT1 facilitates auxin export from the vacuole (Ranocha et al. 2013). In agreement, IAA and related compounds could be isolated from *Arabidopsis* vacuoles (Ranocha et al. 2013). This finding adds another layer of complexity to auxin homeostasis, but the possible role of auxin in the vacuole remains to be demonstrated. Another link has been established between auxin homeostasis and IAA-ALANINE RESISTANT4 (IAR4) that localizes to mitochondria (Quint et al. 2009). *iar4* mutants show auxin-related phenotypes that can be rescued by exogenous auxin application. While free IAA levels are not affected, *iar4* mutants show higher levels of auxin conjugates, suggesting that IAR4 could affect auxin homeostasis.

It remains to be seen whether vacuoles or mitochondria directly or indirectly affect auxin metabolism, but it certainly illustrates the complexity of cellular auxin homeostasis.

5 Concluding Remarks

Auxin transport has important impacts on plant development. While we currently have a good insight into intercellular transport and its importance in plant development, we are still in the early stages of understanding intracellular auxin transport. Compartmentalization of a signaling molecule allows fine-tuning of its cellular activity and/or metabolism. IBA, for example, appears to be a largely inactive auxin molecule that becomes redistributed in plant tissues and spatially converted to an active signal. It is tempting to speculate that IBA converting cells might differ from other tissues in their activity of IBA and/or auxin carriers at peroxisomes. Compartmentalization of IBA metabolism seems to be essential for lateral rooting (De Rybel et al. 2012) and is a stunning example of how environmental signals control postembryonic organ formation (reviewed in Korasick et al. 2013).

IBA metabolism and reversible IAA conjugation to different moieties might appear mechanistically similar, with both leading to IAA inactivation. However, while IBA is actively transported from cell to cell, it remains to be seen whether IAA conjugates are readily transported within plant tissues. Therefore, it is conceivable that IAA conversion to IBA or IAA conjugation exert distinct spatial consequences.

The regulation of auxin conjugation might be linked to the ER (reviewed in Barbez and Kleine-Vehn 2013), but the localization of the molecular players is poorly defined. Apart from its probable role in auxin metabolism, the ER might also be directly involved in auxin signaling. The auxin receptor ABP1 is largely retained in the ER, based on its KDEL retention motif, and only a tiny part of ABP1, constituting its extracellular activity, escapes this regulation (reviewed in Sauer and Kleine-Vehn 2011). It thus seems possible that the ER-localized pool of ABP1 participates in auxin signaling as well (Barbez and Kleine-Vehn 2013). ABP1 in the ER could either directly affect prominent ER functions or provide a signal toward the nucleus via the nuclear envelope. In such a scenario, intracellular auxin transport at the ER would not only affect the rate of compartmentalized auxin metabolism, but might also be a decisive factor for specific auxin signaling events in the ER. Such an intracellular transport mechanism would even allow single cells within a tissue to acquire individual and distinct auxin responses.

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