Polar transport of auxin: carrier-mediated flux across the plasma membrane or neurotransmitter-like secretion?

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Auxin (indole-3-acetic acid) has its name derived from the Greek word *auxein*, meaning 'to increase', and it drives plant growth and development. Auxin is a small molecule derived from the amino acid tryptophan and has both hormone- and morphogen-like properties. Although there is much still to be learned, recent progress has started to unveil how auxin is transported from cell-to-cell in a polar manner. Two recent breakthrough papers from Gerd Jürgens' group indicate that auxin transport is mediated by regulated vesicle trafficking, thus encompassing neurotransmitter-like features.

Auxin is one of the most important molecules regulating plant growth and morphogenesis. At the same time, auxin represents one of the most enigmatic and controversial molecules in plants. Currently, the most popular view is that auxin is a hormone-like substance. However, there are several auxin features and actions that can be much better explained if one considers auxin to be a morphogen-like agent [1–3]. One of the most characteristic features of auxin is its polar cell-to-cell transport [2]. The direction of polar auxin transport is essential for both spatially controlled cell expansion as well as for orientation of planes of division in meristematic cells. No other plant molecule is as important for driving pattern formation and shaping of the whole plant [3]. Moreover, polar auxin transport is sensitive towards the vectorial character of some physical stimuli, especially gravity [4] and light [5]. Environment-induced relocations of auxin allow tight coordination of differential cellular growth along longitudinal cell files (Fig. 1), the typical building blocks of plant tissues [6]. As a result, growing plant organs perform rapid tropic bending in response to changes of environmental cues.

However, a crucial question still remains unanswered. What is the exact nature of the polar cell-to-cell transport of auxin? Importantly, two recent papers from Gerd Jürgens' group illuminate this elusive topic from a new perspective. Firstly, Geldner *et al.* [7] reported that auxin transport inhibitors are, in fact, inhibitors of vesicular recycling of the putative auxin carrier PIN1 [8]. Secondly, the most recent breakthrough article [9] by the same group reveals that PIN1 and the brefeldin-A-sensitive ARF activator GNOM [7,9–11] both localize to endosomes where GNOM mediates sorting of PIN1 from the endosome to the apical plasma membrane. These studies not only shed new light on the polar cell-to-cell transport of auxin but also raise new crucial questions. Where does PIN1 perform its auxin-transporting functions? Does PIN1 transport auxin across the plasma membrane, as all

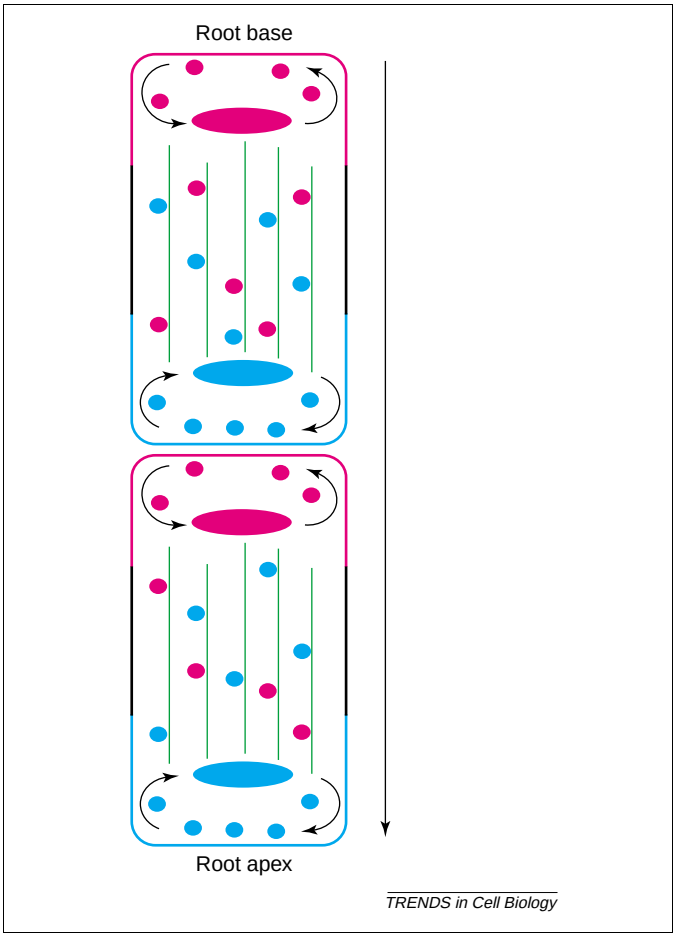

Fig. 1. Schematic overview of two hypothetical root cells of a given cell file. The basal poles are depicted in red and the apical poles in blue. Large arrow indicates the basal-apical polarity axis of the root as well as the direction of the AUX1/PIN1-driven polar auxin transport. The AUX1 influx carrier (red) [22] is localized to the basal pole of root cells [23] and performs brefeldin-A-sensitive recycling between the basal plasma membrane and putative endosome [23]. On the other hand, the PIN1 efflux carrier (blue) [8] localizes to the apical cell pole of root cells, where it accomplishes brefeldin-A-sensitive and GNOM-driven vesicle recycling [7,9,10]. Green lines show longitudinal bundles of F-actin that extend from one pole to the other [24,25] and might contribute to polar auxin transport by guiding endosomal and vesicular trafficking of auxin carriers.

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currently available models propose? Does PIN1 function at internal membranes by loading auxin into endosomes and/or shuttling vesicles? Or do all these membranous sites perform PIN1-mediated auxin transport? Altogether, these and other data seem to support a newly emerging concept that favours a neurotransmitter-like model, with vesicle-trafficking-based secretion of auxin underlying its polar cell-to cell-transport [12–18].

The story of auxin

The story of auxin is full of preconceived ideas and visionary concepts. The history of auxin transport began more than one hundred years ago when Charles Darwin, in his influential book *Power of Movement in Plants*, predicted the existence of a growth-promoting substance that drives polar growth in plants [19]. Darwin postulated that this substance moves from the shoot tip basally and allows tropic bending of shoots in response to physical factors such as light and gravity. The isolation of the proposed hypothetical substance was established some fifty years later [20]. Soon, it became obvious that auxin is transported in a polar fashion from cell-to-cell along the apical-basal plant axis. Nevertheless, molecules and mechanisms driving this transport remained unknown for the next 70 years, although the majority of authors favored a model where auxin carrier-like proteins were inserted into the plasma membrane domains at the opposite ends of elongated cells. In the 1970s, the chemiosmotic hypothesis was postulated proposing that cellular accumulation of auxin is driven by electrical and pH gradients maintained across cell membranes [21], while 'secretion' of auxin was hypothesized to be performed by elusive auxin carriers. After entering cells in its protonated form, or through the action of a saturable uptake carrier, auxin becomes rapidly deprotonated due to the more basic pH values of the cytoplasm. As a result, auxin is effectively trapped inside cells or within vesicular compartments. Consequently, export of auxin from cells requires the action of auxin efflux carriers. In 1996, the identity of the first auxin influx carrier, AUX1, was revealed by Malcolm Bennett and coworkers [22]. Two years later, in a remarkable congruence, four groups simultaneously isolated genes encoding putative auxin efflux carriers, among which PIN1 [8] was the first characterized as a member of a large multigene family (reviewed in [2]).

Do PIN1 and AUX1 transport auxin across the plasma membrane or are they vesicular transporters?

In order to achieve the polar cell-to-cell transport of auxin, cells must be able not only to accumulate auxin but also to export auxin in a polar fashion. One possibility would be that auxin is actively exported by plasma membrane carriers (Fig. 2a). But, until now, physiological experiments have failed to show that this is the case. Another attractive possibility, which has emerged from recent studies by Jürgens and coworkers [4,7,9], is that auxin carriers load auxin into plasma membrane-derived endosomal and endosome-derived recycling vesicles (Fig. 2b). As already mentioned, the auxin transporter PIN1 localizes not only to the plasma membrane but also

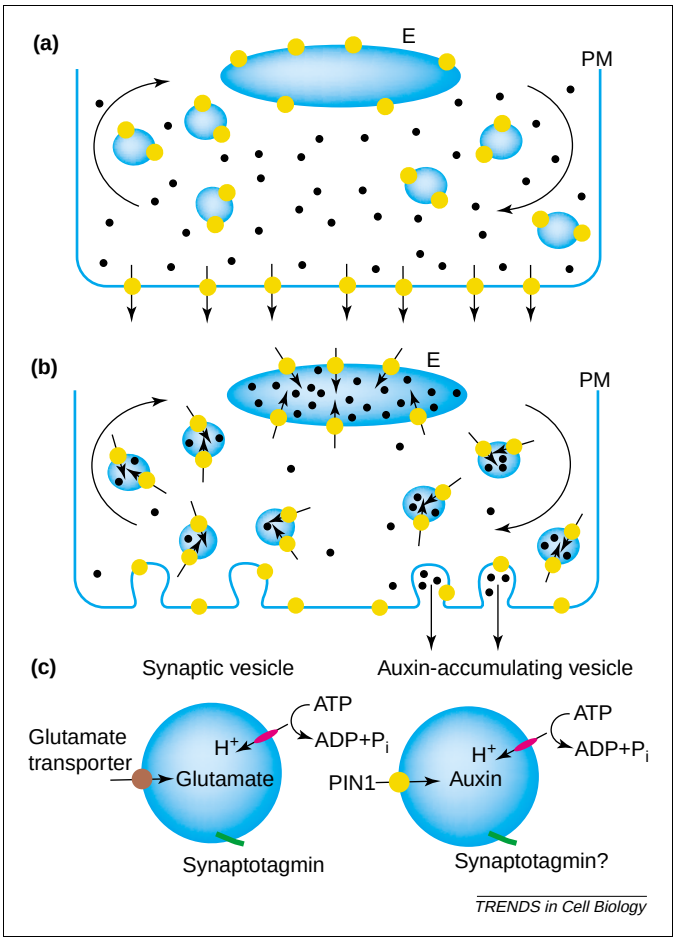

Fig. 2. Detailed view of postulated PIN1 recycling at the apical pole of a hypothetical root cell. PIN1 (yellow circles) recycles between the apical plasma membrane (PM) and endosome (E). In (a), the conventional concept is shown, with PIN1 performing auxin (black dots) export (indicated with arrows) only when inserted into the plasma membrane. This model does not explain the transient nature of the PIN1 localization to the plasma membrane. In (b), the newly emerging model is summarized, based on the current breakthrough papers [7,9] as well as on older data [12–16]. According to this concept, PIN1 is the vesicular transporter that loads auxin (black dots) from the cytoplasm into vesicles and endosomes, while localization of PIN1 to the plasma membrane is only transient owing to its vesicular secretion and rapid retrieval back into internal membranes. In (c), the synaptic vesicle is shown in comparison with a putative auxin-accumulating vesicle. Both types of vesicles accumulate either glutamate [26–28] or auxin [12–15], using a proton gradient as the driving force for crossing their limiting membranes.

to recycling vesicles and endosomes [7,9], and auxin transport inhibitors do not affect auxin transport *per se* but rather inhibit the recycling of PIN1 [7].

In accordance with this vesicle-based scenario, several studies have reported that auxin actively accumulates within plasma membrane-derived vesicles *in vitro* [12–14]. *In vivo*, polar export of auxin could be accomplished via secretory vesicles derived from early endosomes that undergo recycling at the apical plasma membrane (Fig. 2b). This would allow secretion-like export of auxin. Moreover, in order to enhance the polarity of cell-to-cell transport of auxin, it might be that auxin export is driven by vesicle recycling-based exocytosis at one cell pole and that auxin import is closely linked to endocytosis at the opposite cell pole. Therefore, it makes sense that there are two populations of auxin-accumulating vesicles [14]. Phytotropin-sensitive PIN1 vesicles were hypothesized to be localized at one cell pole while phytotropin-insensitive

AUX1 vesicles should be at the opposite cell pole (Fig. 1). In this scenario, PIN1 and AUX1 are not carriers for auxin transport across the plasma membrane but, rather, are vesicular transporters. PIN1 activity is responsible for loading auxin into endosomes and vesicles that shuttle along secretory/recycling pathways (Fig. 2b). In the same manner, AUX1 performs brefeldin-A-sensitive recycling, except that this occurs at the opposite cell pole [23]. These two vesicular pools could be integrated through motile endosomes and the actin cytoskeleton (Fig. 1). Indeed, immunolocalization revealed that actin filaments assemble into longitudinal arrays interconnecting apical/basal end-poles of elongating cells [24,25] and that auxin accumulates within vesicles localized near the plasma membrane [15].

Vesicle trafficking-based cell-to-cell transport of auxin: neurotransmitter-like secretion?

Vesicle-mediated polar auxin transport would closely resemble, at least in some of its aspects, neurotransmitter-based cell-to-cell communication at neuronal synapses (see also [16–18]). Similar to auxin, the neurotransmitter glutamate is a derivative of amino acid metabolism and is actively loaded into endocytic/recycling synaptic vesicles and secreted into the synaptic cleft through regulated exocytosis [26]. Interestingly, both PIN1 and AUX1 carriers are members of the amino acid transporter superfamily, which includes families related to neurotransmitter transporters [27]. In particular, PIN1 is a member of the TC 2.A69 family, which is distantly related to Na^+ symporters from animals (TC 2.28), while AUX1 shows limited homology with VIAAT/VGAT neurotransmitters [28]. Moreover, the ANT1 amino acid transporter can also transport auxin and shows similarity to GABA neurotransmitter transporters [29]. As is the case with auxin carriers, glutamate transporters were also originally considered to be active at the plasma membrane, although physiological studies failed to prove this notion. Only later was it shown that glutamate transporters are vesicular transporters responsible for loading of cytoplasmic glutamate into synaptic vesicles [26]. Similarly to PIN1 and AUX1, vesicular neurotransmitter transporters recycle via vesicular trafficking pathways [26,28]. The universal force behind the loading of neurotransmitter molecules into synaptic vesicles comes from the proton gradient generated by vacuolar (vesicular) ATPases (Fig. 2c), which is also true for auxin loading into plasma-membrane-derived vesicles [12–14].

Concluding remarks

There are several further similarities between polar cell-to-cell transport of auxin in plants and synaptic transmission of signals in animal excitatory tissues. Polar auxin transport requires BIG, a large protein with similarity to calossin [18,30], which controls vesicle recycling during synaptic transmission at neuromuscular junctions [31]. Moreover, synaptic transmission is well known to be driven through highly specialized calcium sensors synchronizing exocytosis and endocytosis according to calcium-mediated signals. One of them is a major synaptic vesicle protein synaptotagmin [32], which not only senses calcium but also allows tight coupling of exocytosis to endocytosis, thus driving synaptic transmission. While synaptotagmins are missing from unicellular yeast, it is intriguing that four synaptotagmin-like genes are present within the *Arabidopsis* genome [33]. Thus, essential pieces of the puzzle seem to fall into their proper places, revealing that higher plants show neuronal-like features in that the end-poles of elongating plant cells [6] resemble chemical synapses. Future studies should focus especially on early postmitotic cells of the transition zone (for roots, see [6]), which not only express high amounts of PIN1 and AUX1 carriers but also polarize their actin cytoskeleton along the apical-basal axis of polarity [24].

In conclusion, besides its hormone- and morphogen-like properties [1–3], the vesicle-based cell-to-cell transport implicates neurotransmitter-like features of auxin too [see also [16–18], making this small but extremely powerful molecule unique in the whole eukaryotic superkingdom.

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Letters

No evidence for PHD fingers as ubiquitin ligases

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The enzymatic conjugation of ubiquitin to eukaryotic proteins typically tags them for degradation by the proteasome, although other signalling roles of protein ubiquitination are beginning to be uncovered. Two different types of ubiquitin ligases are able to mediate the last step of ubiquitination, which is of particular importance as it confers substrate specificity. Proteins of the first group, characterized by the presence of a HECT domain, are true enzymes that catalytically transfer ubiquitin to the target molecule. Members of a second class, characterized by the presence of a complex Zn finger domain termed 'RING finger', act by binding to ubiquitin-conjugating enzymes and bringing them into close contact with the substrate.

In a recent *Opinion* article published in *Trends in Cell Biology*, Coscoy and Ganem proposed a third class of ubiquitin ligases, which rely on PHD fingers instead of RING finger domains [1]. PHD fingers are another type of complex Zn finger, and typically occur in proteins involved in chromatin regulation. The proposal of Coscoy and Ganem is based on two different protein families that reportedly contain PHD fingers and have recently been shown to possess ubiquitin ligase activity: the mitogen-activated protein kinase kinase kinase MEKK1 [2] and a group of viral proteins including MIR1 and MIR2 encoded by the Kaposi sarcoma virus KSHV [3–6]. While there is little doubt that both protein classes can catalyze the transfer of ubiquitin, we disagree with the notion that the Zn fingers in those proteins should be classified as PHD fingers. According to our sequence analysis, both protein classes are slightly atypical members of the RING finger family. Thus, there is no reason to assume that true PHD finger proteins are involved in ubiquitination.

Several sequence-based approaches for the classification of complex Zn fingers are currently in use. The simplest and most accessible one relies solely on the nature and spacing of the cysteine and histidine residues that act as ligands for the Zn(II) ion. This approach, favored by Coscoy and Ganem, predicts that the Zn fingers in MEKK1 and the viral proteins are PHD domains. It should be noted, however, that these easily scorable properties do not always correlate well with Zn finger functionality as there are a number of active RING finger variants with a deviating Cys/His pattern [7].

A fundamentally different approach for Zn finger classification relies on the evolutionary relationship within these families. The general idea is to align the target region to a kind of 'consensus sequence' for established RING fingers or PHD fingers, and to see which alignment score gives a better indication for significant sequence relatedness. In praxis, it is preferable to use sequence profiles or Hidden Markov Models instead of a true consensus sequence as they contain more quantitative information on the relative importance of the residues in the alignment [8]. This concept of protein classification is widely used by databases such as PROSITE [9] and Pfam [10] and has proved very successful in the prediction of protein functionality [11].

Both PROSITE and Pfam already contain entries for RING fingers and PHD fingers, allowing a profile-based classification as described above. We noted that there are several database sequences that test positive for both types of Zn fingers, raising the suspicion that the multiple alignments used for profile construction might have been contaminated by sequences belonging to a different class. To avoid this kind of problem, we constructed a series of generalized profiles [12] specifically dedicated to this classification task by omitting all protein domains with ambiguous status. In addition, we also removed the classification targets themselves and their close relatives from the construction process. As a consequence, the

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