Polar auxin transport: an early invention

Kees J. M. Boot¹,², Kees R. Libbenga¹, Sander C. Hille¹,³, Remko Offringa¹,² and Bert van Duijn¹,⁴,*

¹ Plant Biodynamics Laboratory, Institute Biology Leiden, Leiden University, Sylviuweg 72, 2333 BE Leiden, The Netherlands
² Molecular and Developmental Genetics, Institute Biology Leiden, Leiden University, Sylviuweg 72, 2333 BE Leiden, The Netherlands
³ Mathematical Institute, Leiden University, Niels Bohrweg 1, 2333 CA Leiden, The Netherlands
⁴ Fytagoras, Sylviuweg 72, 2333 BE Leiden, The Netherlands

* To whom correspondence should be addressed. E-mail: bert.vanduijn@fytagoras.nl

Received 11 October 2011; Revised 14 March 2012; Accepted 19 March 2012

Abstract

In higher plants, cell-to-cell polar auxin transport (PAT) of the phytohormone auxin, indole-3-acetic acid (IAA), generates maxima and minima that direct growth and development. Although IAA is present in all plant phyla, PAT has only been detected in land plants, the earliest being the Bryophytes. Charophyta, a group of freshwater green algae, are among the first multicellular algae with a land plant-like phenotype and are ancestors to land plants. IAA has been detected in members of Charophyta, but its developmental role and the occurrence of PAT are unknown. We show that naphthylphthalic acid (NPA)-sensitive PAT occurs in internodal cells of Chara corallina. The relatively high velocity (at least 4–5 cm/h) of auxin transport through the giant (3–5 cm) Chara cells does not occur by simple diffusion and is not sensitive to a specific cytoplasmic streaming inhibitor. The results demonstrate that PAT evolved early in multicellular plant life. The giant Chara cells provide a unique new model system to study PAT, as Chara allows the combining of real-time measurements and mathematical modelling with molecular, developmental, cellular, and electrophysiological studies.

Key words: cytoplasmic streaming, Chara corallina, IAA, polar auxin transport.

Introduction

In higher plants, cell-to-cell polar auxin transport (PAT) of the phytohormone auxin, indole-3-acetic acid (IAA), generates maxima and minima that direct growth and development (Boutte et al., 2007; Zazimalova et al., 2007; Vanneste and Friml, 2009; Grunewald and Friml, 2010; Peer et al., 2011). Although IAA is present in all plant phyla (Cooke et al., 2002; Galván-Ampudia and Offringa, 2007), including members of the Charophyta such as Nitella species (Ross and Reid, 2010), PAT has only been detected in land plants, the earliest being the Bryophytes (Fujita et al., 2008) and most PAT studies have been done in angiosperms and predominantly in Arabidopsis thaliana. These studies indicate that, in angiosperms, auxin is produced in the young developing aerial organs and in shoot apical and root meristems, and also that IAA is transported from shoots to roots by files of specialized parenchyma cells in the vasculature. These transport cells express several classes of auxin influx- and efflux-supporting proteins (Vieten et al., 2007; Petrášek and Friml, 2009). Among the influx carriers are members of the AUXIN RESISTANT1/LIKE AUX (AUX/LAX) proteins, while members of the PIN-FORMED (PIN) and MULTIDRUG RESISTANCE/P-GLYCOPROTEIN ABCD (ABC/PGP) class are involved in auxin flux out of cells. Auxin efflux can be impaired by specific PAT inhibitors such as 1-N-naphthylphthalamic acid (NPA).

In the Chlorophyta unicellular green algae, ABCB/PGP members have been identified by genome analysis, but these algae have no sequence homology to PIN orthologues (Galván-Ampudia and Offringa, 2007). The earliest PIN orthologues, based on sequence data, were found in multicellular green algae Spirogyra pratensis expressed sequence tag libraries (De Smet et al., 2011). There are few PAT measurements in the pteridophyte and bryophyte lower land plants. Polar IAA transport could be measured in elongating rachis from Osmunda cinnamomea fern leaves.
PAT was also detected in sporophytes of the mosses *Bartrania pomiformis*, *Physcomitrella patens*, and *Dawsonia superba*, but not in the gametophores of *P. patens* (Galvan-Ampudia and Offringa, 2007; Fujita et al., 2008; Fujita and Hasebe, 2009; Lau et al., 2009).

Although IAA occurs in members of the Charophyta (Sztein et al., 2000) and Charophyta have a ‘plant-like’ morphology i.e. they have rhizoids, a stem, and branches (Fig. 1), the role of IAA in its growth and development is unresolved. In *Chara*, one study concluded that IAA uptake was not affected by NPA (Dibb-Fuller and Morris, 1992), whereas another reported an enhanced IAA accumulation after addition of NPA to *Chara* explants (Klämbt et al., 1992). These data suggest that proteins involved in IAA transport are present in *Chara*; however, polar cell-to-cell transport of auxin has not been demonstrated in these multicellular algae. The present study investigated the possible presence of PAT in single *Chara* internode cells.

Materials and methods

Materials and growth conditions

Most experiments used *Chara corallina* (Fig. 1), which was originally obtained from Dr. R. J. Reid (University of Adelaide, Australia). *C. corallina* was grown at room temperature in aquaria filled with deionized water and forest soil, under 8/16 light/dark conditions as previously described (Berecki et al., 1999). *Chara vulgaris var. longibracteata* was taken from a pond in the botanical garden of Leiden University, Leiden, The Netherlands.

Polar auxin transport

PAT was performed on excised internodes containing an intact nodal cell on both ends. Measurements were performed in Petri dishes filled with molten paraffin in which grooves between two wells were cut. The grooves were sealed at the donor and receiver ends by embedding the internode just before the nodes in a small amount of silicon grease, covered by a small amount of grafting wax, thus obtaining a water-tight seal (Fig. 2). The wells and bridge compartment were filled with MA medium (half-strength MS Macro I and II, FeNa EDTA, B₃ microelements, 1% sucrose, Masson and Paszkowski, 1992) supplemented with 50 mM MES (pH 4.8). ³H-labelled IAA (specific activity 23 Ci/mmol) was added at a final concentration of 2 × 10⁻⁷ M to either the bridge compartment or the donor well. Radioactivity of the samples was measured in a LKB liquid scintillation counter.

Results and discussion

To explore auxin transport properties of *C. corallina* internodes, we measured auxin transport through single and coupled internodal cells placed in a donor–receiver compartment set up (Fig. 2). ³H-IAA could be added to any of the three compartments (donor, receiver, or bridge) and the appearance of the radio-labelled IAA could be measured in samples from the other compartments.

To determine the transport polarity, radio-labelled auxin was added to the bridge compartment and the amount of IAA secreted into either the donor or receiver compartment was measured. *Chara* internode cells showed a strong polarity with respect to IAA secretion (Fig. 3A). The basal side of the thallus internode (directed towards the rhizoid) exported about 50-times more IAA after 1 h than the distal side (Fig. 3A, about 18 and 0.3 pmol, respectively). After incubation with the PAT inhibitor NPA, IAA export was similar in the basal and distal direction (Fig. 3A). Although
the results were highly reproducible, the amount of IAA accumulating in the receiver wells differed between experiments, with the accumulation ratio between donor and receiver wells ranging from $18 \pm 2.5$ pmol ($n = 2$) to $64 \pm 19$ pmol ($n = 2$). These differences may be due to variations in the internodal developmental stages.

In a classical donor–receiver experiment, IAA accumulation in the receiver compartment was measured after $^3$H-IAA addition to the donor compartment. To confirm the presence of PAT in *Chara*, internode cells were oriented in both directions within the experimental set up. IAA accumulation was only observed with the basal side of the internode in the receiver compartment (Fig. 3B), clearly demonstrating that auxin is polarly transported in internodal cells of *C. corallina*. Similar to land plant stems, auxin was transported from the tip towards the rhizoid. $^3$H-IAA could be detected in the receiver compartment ~45 min after addition of $^3$H-IAA to the donor well. The amount of IAA that was polarly transported in different *Chara* internodes ranged from $1 \pm 0.4$ fmol/min ($n = 5$) to $5.4 \pm 2.8$ fmol/min ($n = 4$).

The same experimental set up was used to measure PAT in two internodes interconnected by a nodium cell. $^3$H-IAA was first detected in the receiver wells ~120 min after its application to the donor wells, which was approximately twice as long as the 45 min taken for single cells (Fig. 3C). These results demonstrate that nodium cells support cell-to-cell PAT through the *Chara* thallus.

Interestingly, $^3$H-IAA taken up from the donor compartment by the *Chara* internode was not only transported to the receiver compartment, but was also significantly lost to the bridge compartment (Fig. 4A). This leaking of IAA may indicate that *Chara* does not possess auxin-influx carrier proteins as observed in higher plants. This is in good agreement with the observation that addition of 2-naphthoxyacetic acid, an auxin influx inhibitor (Lankova et al., 2010), had no effect on the uptake of IAA in *Chara* internode cells (data not shown).
Blasting the available expressed sequence tag data from *Spirogyra* and *Coleochaete* (Timme and Delwiche, 2010) for AUX1/LAX–3 influx carriers did not result in any significant homology. The AUX1/LAX auxin influx carriers in land plants are believed to play an important role as drivers of PAT by taking up leaked auxin and thereby preventing leakage to surrounding cells (Reinhardt et al., 2003). This system may not be very efficient in the aquatic habitat of *C. corallina* where internode cells are in direct contact with a large extracellular volume of water. To test the possibility of IAA loss due to a large extracellular volume, the present study used *C. vulgaris* var. *longibracteata*, in which cortex cells around the large internode cell limit the free space around the cell. This variety had comparable PAT and IAA accumulation in the bridge compartment compared to *C. corallina* (Fig. 4A). Hence, the extra cortical cell layer surrounding the internode cell could not reduce the amount of IAA leakage into the medium, suggesting the lack of influx carriers along the internode cell.

Auxin transport velocity in *Chara* internodes varied between 4–5 cm/h, which was higher than the 1–2 cm/h that is routinely measured in *Arabidopsis* inflorescence stems. This ~4–5 cm/h is probably a minimum estimate taking into account the detection level of the first IAA molecules arriving in the receiver, the loss of auxin into the bridge compartment, and the passage of auxin from the internode through the node into the receiver well. In the bridge experiments, the distance from the bridge compartment to the node into the receiver well was typically ~5 mm (including the seal). Within 1 min, a substantial amount of IAA could be detected in the receiver. Assuming an arrival time of auxin of ~30 seconds, the transport velocity would be 57 cm/h. Therefore, the velocity of PAT in the internode is estimated to be somewhere between 3.6 and 36 cm/h.

Internode cell lengths varied between 3.5–5.0 cm, making it unlikely that auxin is transported intracellularly by simple diffusion. For diffusion the expected transport time of an IAA molecule between the donor and receiver end is at best $L^2/2D$, where $L$ is the length of the internode and $D$ is the diffusion constant of a molecule diffusing in a very thin hollow cylindrical region of the internode cell. The diffusion rate of IAA in agar ($D = 7 \times 10^{-10} \text{ m}^2 \text{s}^{-1}$) (Mitchison, 1980) is a reasonable estimation for diffusion of a 175-Da molecule in a cell (Mika and Poolman, 2011). An IAA molecule would therefore be expected to traverse a 4-cm-long cell in at least 317 h. Hence it is unlikely that, the PAT velocities measured are caused by diffusion.

Cytoplasmic streaming could be an alternative candidate mechanism for the transport of intracellular auxin in *Chara* internodes (Verchot-Lubicz and Goldstein, 2010). The present study measured velocities of ~12–20 cm/h (data not shown) for cytoplasmic streaming in *Chara*. Cytochalasin H, a cytoplasmic streaming inhibitor, has been extensively studied in *Chara* (Foissner and Wasteneys, 2007). In the present experiments, cytochalasin H inhibited cytoplasmic streaming in *Chara* internodes within 5 min, and replacement with buffer reinitiated cytoplasmic streaming after 15 min, with initial velocity regained after 30 min. The effect of cytochalasin H on PAT in *Chara* internodes was investigated by either pre-incubating the internodes with cytochalasin H within the bridge compartment or adding the inhibitor to the bridge compartment after PAT had been ongoing for 270 min. In both cases, no significant effect of cytochalasin H on PAT was observed (Fig. 4B), which is in agreement with the slightly reduced PAT rates using cytochalasin B in oat and maize coleoptiles (Cande et al., 1973). However, cytochalasin H in *Chara* has been reported to only inhibit vesicle transport at the cortical part of the cytoplasm. Inhibition of cytoplasmic streaming of the subcortical cytoplasm takes several days of treatment with cytochalasin H before any effects can be measured (Foissner and Wasteneys, 2007). This subcortical cytoplasmic streaming can reach velocities of 18–36 cm/h, making it a good candidate for the observed intracellular auxin transport. The present study also tested the effect of cytochalasin H on
the subcortical cytoplasmic streaming and found that it could be completely and reversibly inhibited by the addition of the inhibitor. Therefore, cytoplasmic streaming is an unlikely mechanism for intracellular auxin transport in Chara cells. It remains an intriguing question as to what the driving force for this transport in Chara is.

The accumulation curve of IAA in the receiver in a classical donor–receiver transport experiment (Fig. 3B) can be described by the solution to a system of convection–diffusion equations with physiologically feasible values for the parameters. The corresponding accumulation curve for a bridge experiment (Fig. 3A), however, was quite different. During the first minute, the flux into the receiver was as high as ~73 mol/s on average, which within a few minutes levelled off to a steady-state value of ~2.5 fmol/s. Up till now, this dynamic behaviour could not be reconciled with the system of convection–diffusion equations describing the classical donor–receiver experiments. One interpretation of the data could be that there is a rapid downregulation of the transporters themselves or the activity of the proteins that are involved in the polar efflux of auxin. A systematic mathematical analysis of the transport data from specifically designed experiments is in progress.

Conclusion

Here we show that PAT is present in Charophyta, an evolutionary ancestor of higher plants (Finet et al., 2010; Timme et al., 2012). The ability to measure PAT at the cellular level provides a unique opportunity to further analyse PAT-related parameters such as membrane potential, cytosolic calcium, and pH levels that drive IAA transport inside the cell. It will be important to determine the proteins responsible for PAT in Chara and their relationship to the well-known PIN and ABCD/PGP classes of PAT efflux proteins found in higher plants. It will also be interesting to test the hypothesis that Chara lacks AUX1/LAX auxin influx proteins. Understanding the dynamics of PAT in an experimental unicellular system is easier than in a complex cell system such as Arabidopsis inflorescence stems. The former is better suited for mathematical modelling and analysis. Moreover, the extreme proportions of the Chara internode cell and the experimental set up reveal details of IAA transport that are relevant in reconstructing the transport mechanism and that are hard to observe in Arabidopsis. The similarity in PAT between Chara and Arabidopsis inflorescence stems indicates that PAT is a critical evolutionary early invention of multicellular plants that enables intercellular communication and directional growth.

Acknowledgements

This work was supported by TTI-Green Genetics (KJMB). The authors thank R. van Vugt for C. vulgaris var. longibracteata.

References

Berecki G, Varga Z, van Iren F, van Duijn B. 1999. Anion channels in Chara corallina tonoplast membrane: calcium dependence and rectification. Journal of Membrane Biology 172, 159–168.

Boutte Y, Ikeda Y, Grebe M. 2007. Mechanisms of auxin-dependent cell and tissue polarity. Current Opinion in Plant Biology 10, 616–623.

Cande WZ, Goldsmith MHM, Ray PM. 1973. Polar auxin transport and auxin-induced elongation in the absence of cytoplasmic streaming. Planta 111, 279–296.

Cooke TJ, Poli DB, Sztein AE, Cohen JD. 2002. Evolutionary patterns in auxin action. Plant Molecular Biology 49, 319–338.

De Smet I, Voß U, Lau S, et al. 2011. Unraveling the evolution of auxin signalling. Plant Physiology 155, 209–221.

Dibb-Fuller JE, Morris DA. 1992. Studies on the evolution of auxin carriers and phytotropin receptors: transmembrane auxin transport in unicellular and multicellular Chlorophyta. Planta 186, 219–226.

Finet C, Timme RE, Delwiche CF, Marletaz F. 2010. Multigene phylogeny of the green lineage reveals the origin and diversification of land plants. Current Biology 20, 2217–2222.

Foisner I, Wastenyes GO. 2007. Wide-ranging effects of eight cytochalasins and latrunculin A and B on intracellular motility and actin filament reorganization in Characean internodal cells. Plant and Cell Physiology 48, 585–597.

Fujita T, Hasebe M. 2009. Convergences and divergences in polar auxin transport and shoot development in land plant evolution. Plant Signaling and Behavior 4, 313–315.

Fujita T, Sakaguchi H, Hiwatashi Y, Wagstaff SJ, Ito M, Deguchi H, Sato T, Hasebe M. 2008. Convergent evolution of shoots in land plants: lack of auxin polar transport in moss shoots. Evolution and Development 10, 176–186.

Galvan-Ampudia C, Offringa R. 2007. Plant evolution: AGC kinases tell the auxin tale. Trends in Plant Science 12, 541–547.

Grunewald W, Friml J. 2010. The march of the PINs: developmental plasticity by dynamic polar targeting in plant cells. The EMBO Journal 29, 2700–2714.

Klämbt D, Knauth B, Dittmann I. 1992. Auxin dependent growth of rhizoids of, Chara globularis. Physiologia Plantarum 85, 537–540.

Lankova M, Smith RS, Pesek B, et al. 2010. Auxin influx inhibitors 1-NOA, 2-NOA, and CHPAA interfere with membrane dynamics in tobacco cells. Journal of Experimental Botany 61, 3589–3598.

Lau S, Shao N, Bock R, Jurgens G, De Smet I. 2009. Auxin signaling in algal lineages: fact or myth? Trends in Plant Science 14, 182–188.

Masson J, Paszkowski J. 1992. The culture response of Arabidopsis thaliana protoplasts is determined by the growth conditions of donor plants. The Plant Journal 2, 829–833.

Mika JT, Poolman B. 2011. Macromolecule diffusion and confinement in prokaryotic cells. Current Opinion in Biotechnology 22, 117–126.

Mitchison GJ. 1980. The dynamics of auxin transport. Proceedings of the Royal Society of London. Series B, Biological Sciences 209, 489–511.

Peer WA, Blakeslee JJ, Yang H, Murphy AS. 2011. Seven things we think we know about auxin transport. Molecular Plant 4, 487–504.
Petrašek J, Friml J. 2009. Auxin transport routes in plant development. Development 136, 2675–2688.

Reinhardt D, Pesce E-R, Stieger P, Mandel T, Baltensperger K, Bennett M, Traas J, Friml J, Kuhlemeier C. 2003. Regulation of phyllotaxis by polar auxin transport. Nature 426, 255–260.

Ross JJ, Reid JB. 2010. Evolution of growth-promoting plant hormones. Functional Plant Biology 37, 795–805.

Steves TA, Briggs WR. 1960. Morphogenetic studies on Osmudacinnamomea L. The auxin relationships of expanding fronds. Journal of Experimental Botany 11, 45–67.

Sztein AE, Cohen JD, Cooke TJ. 2000. Evolutionary patterns in the auxin metabolism of green plants. International Journal of Plant Sciences 161, 849–859.

Timme RE, Bachvaroff TR, Delwiche CF. 2012. Broad phylogenomic sampling and the sister lineage of land plants. PLoS One 7, e29696.

Timme RE, Delwiche CF. 2010. Uncovering the evolutionary origin of plant molecular processes: comparison of Coleochaete (Coleochaetales) and Spirogyra (Zygnematales) transcriptomes. BMC Plant Biology 10, 96.

Vanneste S, Friml J. 2009. Auxin: a trigger for change in plant development. Cell 136, 1006–1016.

Verchot-Lubicz J, Goldstein RE. 2010. Cytoplasmic streaming enables the distribution of molecules and vesicles in large plant cells. Protoplasma 240, 99–107.

Vieten A, Sauer M, Brewer PB, Friml J. 2007. Molecular and cellular aspects of auxin-transport-mediated development. Trends in Plant Science 12, 160–168.

Zazimalova E, Krecek P, Skupa P, Hoyerova K, Petrasek J. 2007. Polar transport of the plant hormone auxin—the role of PIN-FORMED (PIN) proteins. Cell and Molecular Life Sciences 64, 1621–1637.