Tansley insight

Auxin fluxes through plasmodesmata

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Contents

Summary

Characterising the processes that control auxin dynamics is essential to understanding how auxin regulates plant development. Over recent years, several studies have investigated auxin diffusion through plasmodesmata, characterising this cell-to-cell diffusion and demonstrating that it affects auxin distributions. Furthermore, studies have shown that plasmodesmatal auxin diffusion affects developmental processes, including phototropism, lateral root emergence and leaf hyponasty. This short Tansley Insight review describes how these studies have contributed to our understanding of auxin dynamics and discusses potential future directions in this area.

I. Introduction

The hormone auxin controls many aspects of plant development, including organ initiation, tropic responses and growth (Benjamins & Scheres, 2008). Key to this regulation is the dynamic auxin distribution. Many studies have investigated how the auxin distribution is created by the presence of membrane proteins PIN, AUX1/LAX and ABCB, which create auxin maxima by mediating active transport across specific cell membranes (Reinhardt *et al.*, 2003). However, recently, several studies have investigated an additional auxin transport pathway – passive diffusion via plasmodesmata. Plasmodesmata are membrane-lined channels that are embedded within the cell wall and connect the cytoplasms of adjacent cells to form what is known as the symplast (see (Faulkner, 2018; Sager & Lee, 2018; Li *et al.*, 2021) for recent reviews on plasmodesmata). Plasmodesmata are known to play a

key role in cell-to-cell communication and signalling by enabling water, nutrients, and other small molecules to diffuse between adjacent cells. Recent studies have revealed that auxin diffusion through plasmodesmata contributes to auxin distribution, and that manipulating this transport pathway impacts auxin-related phenotypes. This short Tansley Insight review describes these studies and discusses future directions in this area.

II. Plasmodesmatal auxin diffusion contributes to auxin distribution

Auxin movement through plasmodesmata is thought to be a passive process, with the auxin flux proportional to the difference in auxin concentration between the neighbouring cells. This passive diffusion smooths out spatial variations in auxin concentration, and so its role has been somewhat unintuitive, given such diffusion

may diminish the auxin maxima that have been shown to be important for many developmental processes.

Quantifying the rate of auxin diffusion through plasmodesmata was an essential step in determining how plasmodesmatal diffusion combines with the active transport mediated by the PIN and AUX1/LAX carriers (see Box 1 and Fig. 1a for a summary of the components of auxin transport). Rutschow *et al.* (2011) used fluorescent recovery after photobleaching to quantify plasmodesmatal permeability to small molecules in the Arabidopsis root meristem. By interpreting their data using a mathematical model, they found an effective plasmodesmatal permeability of $6–8.5 \ \mu m \ s^{-1}$, which is of similar magnitude to permeability estimates for the carriers (i.e. $2.8 \ \mu m \ s^{-1}$ for PINs (Kramer, 2004; Kramer *et al.*, 2011) and $1.5 \ \mu m \ s^{-1}$ for AUX1 (Rutschow *et al.*, 2014)).

How does plasmodesmatal diffusion combine with the PIN-mediated polar efflux? In a single cell file, Mitchison (1980) showed that cell-to-cell diffusion retards the auxin velocity created by the PINs. Using their permeability measurements, Rutschow *et al.* (2011) calculated that plasmodesmatal diffusion reduces the auxin velocity by *c.* 34% (for cell lengths of 10 μm).

Understanding of how polar PIN efflux and plasmodesmatal diffusion combine can also be gained by considering a 2D tissue (Fig. 2a–d; Supporting Information Notes S1). Plasmodesmatal diffusion enables auxin to spread in all directions (Fig. 2b), whereas the polar PINs create a directed transport (Fig. 2c). With both these processes, plasmodesmatal diffusion reduces the auxin velocity created by the PINs and enables auxin to move between adjacent cell files (Fig. 2d).

AUX1/LAX influx carriers also play a major role in creating auxin distributions; in the root apex, for example, modelling has suggested that AUX1/LAX controls which tissues have high auxin levels, whereas PINs control the auxin-transport direction within these tissues (Band *et al.*, 2014). Motivated by the measurements of Rutschow *et al.* (2011), Mellor *et al.* (2020) used a systems approach to analyse how plasmodesmatal diffusion contributes to the root-apex auxin distribution created by the PIN and AUX1/LAX carriers (Fig. 1b). Simulations of auxin dynamics using a realistic multicellular template and data on plasmodesmatal permeability (Rutschow *et al.*, 2011) and density (Zhu *et al.*, 1998) revealed that plasmodesmatal diffusion modifies the auxin distribution. AUX1 influx carriers in the outer root layers create high auxin levels in these layers. The model showed that plasmodesmatal auxin diffusion enabled auxin reflux from these AUX1-expressing outer layers, which have high auxin levels, towards the stele, and thus, plasmodesmata enable auxin to bypass the apoplast, where AUX1 uptake would return auxin to the outer layers (Fig. 1b). Hence, plasmodesmatal diffusion and AUX1 influx work together to create a reflux loop that increases the total root-tip auxin (Mellor *et al.*, 2020).

III. Plasmodesmatal diffusion is modified via callose

The rate of plasmodesmatal diffusion between adjacent cells depends on both the plasmodesmata density and the rate of diffusion through individual plasmodesmata. With uniform plasmodesmata, plasmodesmatal diffusion enables auxin to spread evenly through a tissue (Fig. 2b). However, in plants both the number of plasmodesmata and their properties vary both spatially and temporally, affecting the diffusion rate between neighbouring cells. The resulting transport pathway is highly regulated and dynamic.

Plasmodesmatal fluxes can be modified by callose, which reduces plasmodesmatal permeability by physically restricting the pore (Amsbury *et al.*, 2018). Once synthesised, callose deposits within minutes (Jaffe & Leopold, 1984), enabling a rapid control mechanism. Several genes have been shown to regulate plasmodesmatal callose: GLUCAN SYNTHASE LIKE 8 (GSL8) and CALLOSE SYNTHASE 3 (CALS3) regulate callose synthesis (Chen *et al.*, 2009; Vatén *et al.*, 2011; Han *et al.*, 2014); beta-1,3-glucanase (PdBG) contributes to callose degradation (Iglesias & Meins, 2000; Levy *et al.*, 2007); PLASMODESMATA CALLOSE BINDING1 (PDCB1) binds callose in the cell wall around the plasmodesmata (Simpson *et al.*, 2009) and PDLP5 stimulates callose deposition (Lee *et al.*, 2011; Wang *et al.*, 2013). Mutants in several of these gene families have been shown to modify plasmodesmatal diffusion (Simpson *et al.*, 2009; Rutschow *et al.*, 2011) and affect auxin distributions (Han *et al.*, 2014; Gao *et al.*, 2020; Mellor *et al.*, 2020; Sager *et al.*, 2020); thus, these genetic approaches have been key tools in elucidating how plasmodesmatal diffusion contributes to auxin-related phenotypes (as described in sections IV, V and VI below).

IV. Callose-mediated reduction in plasmodesmatal auxin diffusion is essential to hypocotyl phototropism

That auxin movement through plasmodesmata impacts plant phenotype was first demonstrated in the context of hypocotyl phototropism by Han *et al.* (2014). Here, light induces tropic bending due to auxin accumulation on the shaded side. Interestingly, Han *et al.* (2014) observed that tropic stimulation leads to callose deposition at the shaded side, where auxin accumulates.

Box 1   Summary of the key components of auxin transport

Auxin movement through plant tissues occurs through a range of different transport processes (also see Fig. 1a):
- Protonated auxin passively diffuses across plasma membranes.
- AUX1/LAX membrane proteins mediate the influx of anionic auxin from the apoplast to the cell cytoplasms.
- PIN membrane proteins mediate the efflux of anionic auxin from the cell cytoplasms to the apoplast.
- ABCB membrane proteins mediate auxin transport across cell membranes, predominantly thought to be efflux from cytoplasms to apoplast.
- Auxin within the apoplast passively diffuses.
- Auxin passively diffuses through plasmodesmata between adjacent cell cytoplasms.

The overall auxin distribution and flux pattern depends on the combined effects of these processes.
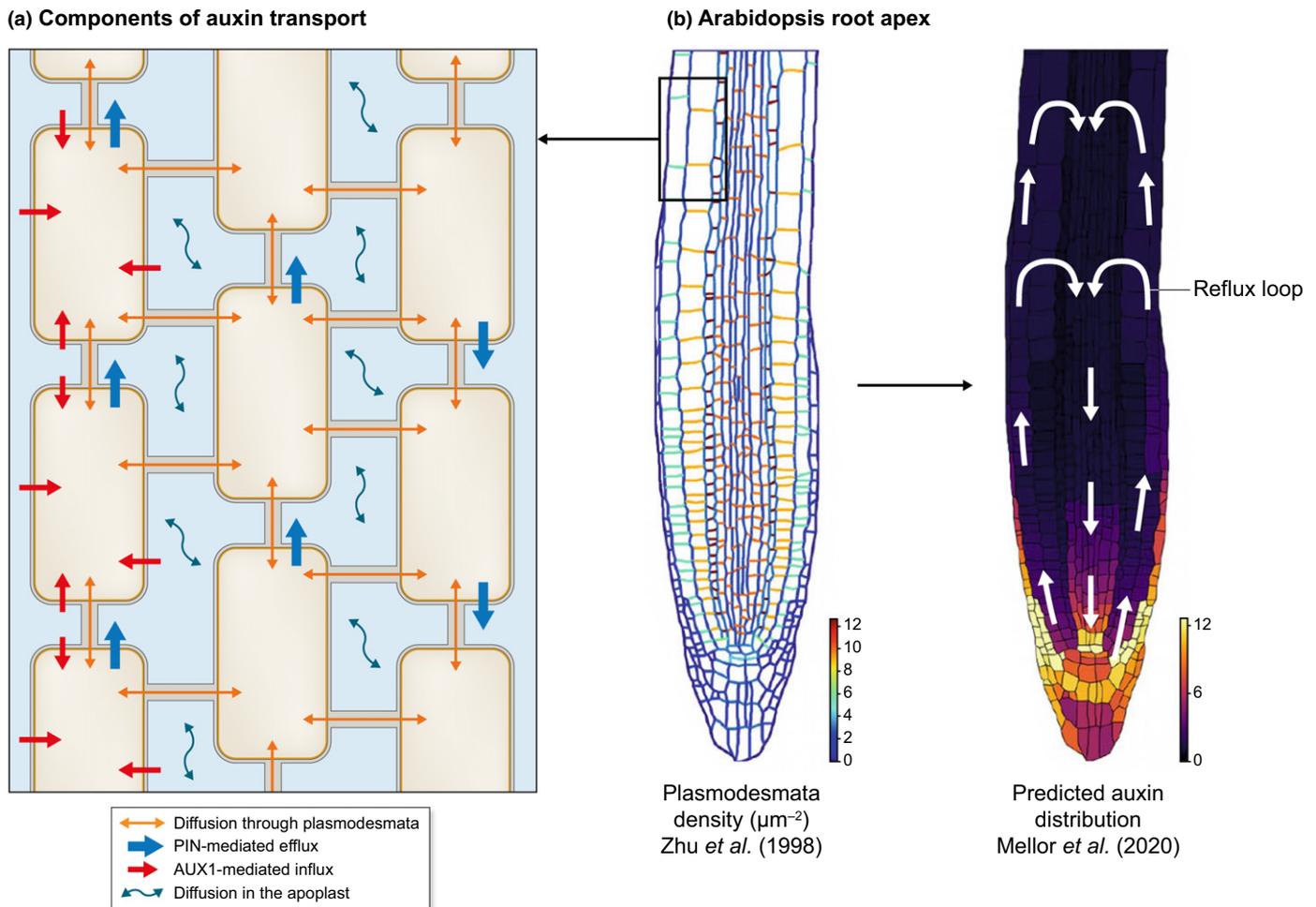

(a) Components of auxin transport

Diffusion through plasmodesmata
PIN-mediated efflux
AUX1-mediated influx
Diffusion in the apoplast

(b) Arabidopsis root apex

Reflux loop

Plasmodesmata
density (µm^{-2})
Zhu *et al.* (1998)

Predicted auxin
distribution
Mellor *et al.* (2020)

Fig. 1 Summary of the key components of auxin transport (a) and their predicted role in the Arabidopsis root apex (b). Panel (b) is adapted from Mellor *et al.* (2020), which simulates auxin transport within a realistic multicellular root apex using data on the plasmodesmatal density distribution from Zhu *et al.* (1998) and plasmodesmatal permeability from Rutschow *et al.* (2011). Plasmodesmatal diffusion enables auxin reflux from the outer layers to the stele (as shown with white arrows in (b)), bypassing the apoplast where AUX1 mediates auxin influx back into the outer layers.

If callose deposition does not occur, as observed using an inducible *gsl8* RNAi line or callose synthesis inhibitors, the auxin gradient across the hypocotyl does not form and bending does not occur. The results suggested a feedback mechanism whereby a small increase in auxin on the shaded side upregulates GSL8 (in an ARF7-dependent manner), promoting callose synthesis and reducing diffusion through plasmodesmata. Thus, by upregulating GSL8, auxin can restrict its own movement, maintaining the auxin gradient across the hypocotyl which is essential to mediating the tropic bending response.

V. Callose-mediated reduction in plasmodesmatal auxin diffusion controls the timing of lateral root emergence

Auxin maxima are also essential to lateral root development, and here, dynamic control of symplastic connectivity via callose regulation is also required (Benitez-Alfonso *et al.*, 2013; Maule *et al.*, 2013; Sager *et al.*, 2020). Initial studies demonstrated that

cells of lateral root primordia are connected via plasmodesmata to the pericycle at initial stages of development, but gradually become isolated (Benitez-Alfonso *et al.*, 2013; Maule *et al.*, 2013); precisely how this gradual isolation affects the auxin dynamics that underpin lateral root development promises to be an interesting topic for future work.

The role of plasmodesmatal auxin diffusion has, however, been recently studied during lateral root emergence (Sager *et al.*, 2020). During this process, cell-wall separation in the overlying layers is triggered by auxin released from the tip of growing primordia (Swarup *et al.*, 2008). Sager *et al.* (2020) revealed that regulation of plasmodesmatal auxin diffusion controls the timing of this process. Auxin was shown to induce PDLP5 in the overlying cells, which restricts plasmodematal diffusion by increasing callose deposition (Lee *et al.*, 2011; Wang *et al.*, 2013). Studying knockout and over-expression lines revealed that PDLP5 delays lateral root emergence in the later stages by reducing auxin diffusion in the overlying tissues, which restricts the auxin maxima to only a few cells (Sager *et al.*, 2020). Thus, with parallels to the phototropism study of Han
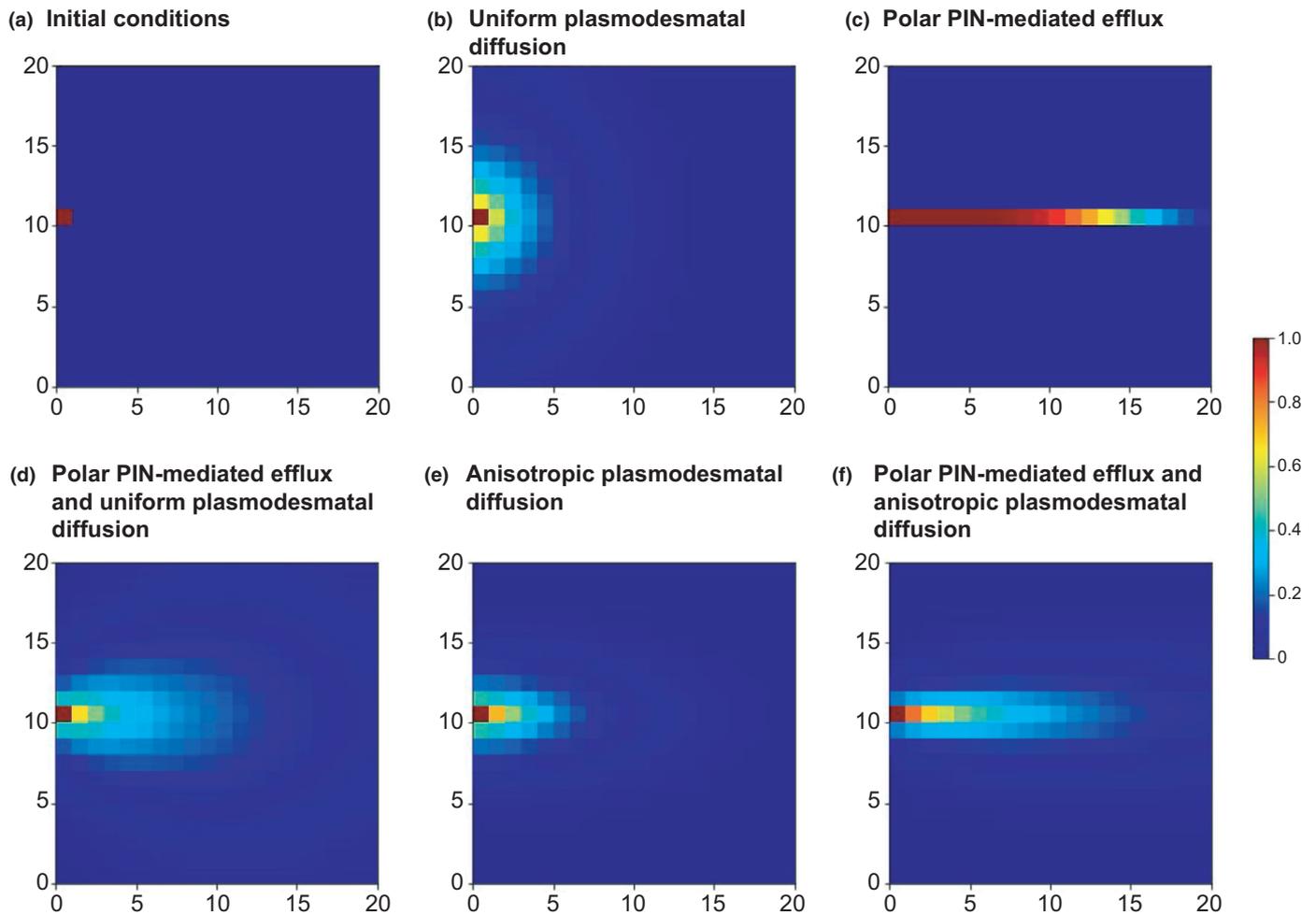
Fig. 2 Illustrative simulations showing the effect of plasmodesmatal auxin diffusion and polar PIN-mediated efflux on the spread of auxin in a 2D grid of square cells. PINs are located on the right-hand face of each cell. The flux from cell i,j to its horizontal neighbour, to the right, is $Q_h = P_{PIN}c_{ij} + P^h_{plas}(c_{ij} - c_{(i+1)j})$, and to its vertical neighbour, above, is $Q_v = P^v_{plas}(c_{ij} - c_{i(j+1)})$ where c_{ij} denotes the cell's auxin concentration, P_{PIN} is the permeability associated with the PIN-mediated flux, and P^h_{plas} and P^v_{plas} are the permeabilities associated with the plasmodesmatal diffusion in the horizontal and vertical directions, respectively. A single cell has a fixed concentration equal to 1 (as shown in (a)). (a) Initial conditions at $t = 0$; (b) uniform plasmodesmatal diffusion, $P_{PIN} = 0$, $P^h_{plas} = P^v_{plas} = 2.8\,\mu m\,s^{-1}$; (c) polar PIN-mediated efflux, $P_{PIN} = 2.8\,\mu m\,s^{-1}$, $P^h_{plas} = P^v_{plas} = 0$; (d) polar PIN-mediated efflux and uniform plasmodesmatal diffusion, $P_{PIN} = P^h_{plas} = P^v_{plas} = 2.8\,\mu m\,s^{-1}$; (e) anisotropic plasmodesmatal diffusion (channelling), $P_{PIN} = 0$, $P^h_{plas} = 4.91\,\mu m\,s^{-1}$, $P^h_{plas} = 0.68\,\mu m\,s^{-1}$, based on values in Gao *et al.* (2020); (f) polar PIN-mediated efflux and anisotropic diffusion, $P_{PIN} = 2.8\,\mu m\,s^{-1}$, $P^h_{plas} = 4.91\,\mu m\,s^{-1}$, $P^h_{plas} = 0.68\,\mu m\,s^{-1}$. Simulation results are shown at $t = 50\,s$, and for cell lengths and widths of 10 μm. Concentrations are assumed to be uniform within each cell and apoplastic diffusion is omitted.

et al. (2014) described in section IV, above, plasmodesmatal regulation forms a feedback loop whereby auxin restricts its own movement in order to create and maintain an auxin maximum.

VI. Anisotropic plasmodesmatal diffusion affects auxin dynamics in leaves and roots

Differences in plasmodesmatal permeability on different cell faces can create anisotropic diffusion or channelling (Fig. 2e,f). Such a phenomenon was recently discovered by Gao *et al.* (2020) in Arabidopsis leaves, where fluorescent tracer experiments revealed more rapid diffusion in the longitudinal direction (along the midrib) than in the transverse direction. Callose staining revealed that these differences were caused by increased GSL8-mediated callose deposition in plasmodesmata connecting neighbouring cells in the transverse direction, resulting in plasmodesmata that are partly blocked. Both computational modelling and auxin applications revealed that the resulting anisotropic diffusion increases the rate at which auxin from the leaf tip reaches the petiole. The callose distribution and resulting anisotropic auxin diffusion was shown to increase the leaf hyponasty response, whereby auxin applied at the leaf tip triggers upward movement of the leaf position, which is physiologically beneficial for shade avoidance.

Interestingly, anisotropic plasmodesmatal diffusion is also thought to be present in the Arabidopsis root tip, due to plasmodesmatal density being higher between adjacent cells in each tissue layer than between cells from different tissue layers (Zhu *et al.*, 1998, Fig. 1b). Simulations from Mellor *et al.* (2020),

suggested that this plasmodesmatal distribution is important for creating the root-apex auxin distribution.

VII. Auxin feeds back on its own plasmodesmatal diffusion

With numerous genes regulating plasmodesmatal fluxes via callose, there are many factors that may control plasmodesmatal diffusion. Key to the studies on plasmodesmatal auxin fluxes during phototropism and lateral root emergence (Han *et al.*, 2014; Sager *et al.*, 2020) was the observation that that auxin feeds back on its own transport, by increasing callose levels to restrict its own movement. These findings addressed the long-held concern that auxin diffusion through plasmodesmata would dissipate the auxin maxima that are created by the carrier-mediated transport and that are essential for numerous developmental processes.

However, other studies have not found such feedback regulation, suggesting that whether auxin controls its own plasmodesmatal diffusion may depend on the plant organ. Measurements in both the Arabidopsis root meristem and leaf found that the effective plasmodesmatal permeability was not affected by exogenous auxin treatments (Rutschow *et al.*, 2011; Gao *et al.*, 2020). Furthermore, support for differences in feedback regulation between organs comes from a root-specific transcriptomics data set (Voß *et al.*, 2015) which revealed that auxin treatment did not change the expression of some of the callose regulators (GSL8, PdBG or PDCB1) (Mellor *et al.*, 2020), in contrast to the auxin induction of GSL8 observed in the hypocotyl (Han *et al.*, 2014). These studies, however, all investigated responses to exogenous auxin applications, and therefore do not exclude the possibility that lowering auxin concentration may affect callose levels in these organs. Furthermore, these studies used applications of indole-3-acetic acid (IAA), which may be affected by auxin redistribution by auxin carriers.

Additional levels of feedback have also been discovered, which would further modify auxin dynamics. For example, in the root meristem, Y. Liu *et al.* (2017) detected a reduction in auxin biosynthesis genes after disrupting symplastic transport into the quiescent centre (QC), whereas Wu *et al.* (2016) observed ectopic PIN2 expression after inducing callose synthesis in the endodermis. The relative influence of these processes and the associated feedbacks remains to be unpicked.

VIII. Conclusions and future outlook

Recent studies have demonstrated clearly that diffusion through plasmodesmata is a key component of the auxin transport machinery, both affecting the auxin distribution and, most importantly, contributing to plant phenotypes (Paterlini, 2020). Manipulating auxin diffusion through plasmodesmata (using lines that regulate callose levels) has been shown to affect auxin distributions, phototropism, lateral root emergence and leaf hyponasty.

The role of plasmodesmatal auxin fluxes was made clearer by several recent developments. Several studies were enabled by the DII-VENUS auxin sensor, which, being closely related to auxin

levels, enables quantification and visualisation of auxin dynamics (Brunoud *et al.*, 2012). This sensor was essential, for example, for deducing the role of plasmodesmatal auxin diffusion in shoot tropisms (Han *et al.*, 2014) and the root apex (Mellor *et al.*, 2020). Furthermore, computational modelling was used effectively, from the early analysis of Mitchison (1980), to more recent studies that calculated permeability estimates from fluorescence data (Rutschow *et al.*, 2011) and predicted how plasmodesmatal diffusion contributes to organ-scale distributions and fluxes (Gao *et al.*, 2020; Mellor *et al.*, 2020). Thus, this area has very much grown from recent developments in molecular biology and systems biology techniques.

The recent studies described in this review have cemented plasmodesmatal diffusion as a key process contributing to auxin distributions and phenotypes. The roles of plasmodesmatal diffusion in other auxin-mediated processes, such as during phyllotaxis in the shoot apical meristem, promise to be interesting topics for future work (see Kitagawa & Jackson, 2017 for a recent review on plasmodesmata in the shoot apical meristem). Furthermore, other hormones will also diffuse through plasmodesmata, and studies have shown that plasmodesmatal callose levels are regulated by several other hormones – abscisic acid (ABA; J. Liu *et al.*, 2017), gibberellic acid (Rinne *et al.*, 2011) and salicylic acid (Wang *et al.*, 2013) – providing an additional mechanism of crosstalk between the hormone pathways. Plasmodesmatal permeability is also affected by environmental conditions such as low temperature, light, osmotic stress, and toxic metals (reviewed in Sager & Lee, 2014), and studying plasmodesmatal auxin diffusion in these conditions may provide further examples of mechanisms whereby environmental conditions affect auxin dynamics to mediate developmental plasticity. A recent study suggested that plasmodesmatal regulation by light is gated by the circadian clock (Brunkard & Zambryski, 2019), which could potentially lead to oscillations in auxin dynamics, for example, to influence circadian rhythms in growth dynamics.

While this review focussed on auxin-related phenotypes, it is important to emphasise that plasmodesmatal regulation will affect transport of numerous other substances, including nutrients, water, signalling molecules, mRNA, viruses and other hormones. Callose deposition reduces the physical space available for molecules to diffuse through, and its effect on diffusion will depend on both the size of the molecule and the level of restriction. As auxin is a small molecule, callose deposition is thought to reduce the auxin flux (Rutschow *et al.*, 2011). However, substantial callose deposition can result in cells being completely isolated (in what are called 'symplastic domains') and callose deposition can physically prevent the movement of larger molecules, whilst allowing smaller molecules to diffuse (as characterised by the size exclusion limit) (Faulkner, 2018). Thus, plasmodesmatal auxin diffusion and auxin regulation of plasmodesmatal permeability are part of a very complex network of cross-talks between different pathways. While callose deposition may be beneficial for the distribution of one molecule, it will also reduce the diffusion of other molecules. For example, in several studies, auxin regulation of plasmodesmatal callose was found to be essential in creating create auxin gradients (Han *et al.*, 2014; Sager *et al.*, 2020); however, such callose

deposition will also reduce the diffusion of other molecules. Whether the callose deposition also has a detrimental effect on growth by isolating cells from sources of sugar and other nutrients remains an open question. Unpicking the coupling between different pathways presents an exciting challenge for future research.

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Supporting Information

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Notes S1 Python code used to produce the simulations shown in Fig. 2.

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