Manipulation of Auxin Transport in Plant Roots during 
*Rhizobium* Symbiosis and Nematode Parasitism

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The plant rhizosphere harbors many different microorganisms, ranging from plant growth–promoting bacteria to devastating plant parasites. Some of these microbes are able to induce de novo organ formation in infected roots. Certain soil bacteria, collectively called rhizobia, form a symbiotic interaction with legumes, leading to the formation of nitrogen-fixing root nodules. Sedentary endoparasitic nematodes, on the other hand, induce highly specialized feeding sites in infected plant roots from which they withdraw nutrients. In order to establish these new root structures, it is thought that these organisms use and manipulate the endogenous molecular and physiological pathways of their hosts. Over the years, evidence has accumulated reliably demonstrating the involvement of the plant hormone auxin. Moreover, the auxin responses during microbe-induced de novo organ formation seem to be dynamic, suggesting that plant-associated microbes can actively modify their host’s auxin transport. In this review, we focus on recent findings in auxin transport mechanisms during plant development and on how plant symbionts and parasites have evolved to manipulate these mechanisms for their own purposes.

INTRODUCTION

Terrestrial plants develop an elaborate root system that optimizes their anchorage and the uptake of water and nutrients. Moreover, the plant root system must show extensive developmental plasticity to respond to ever-changing environmental conditions. Hard objects in the soil force the root to redirect its growth (thigmotropism) (Massa and Gilroy, 2003), while a suboptimal concentration of essential minerals and nutrients stimulates root branching (Lopez-Bucio et al., 2002). These processes are to a great extent orchestrated by the phytohormone auxin and in particular by active cell-to-cell transport of auxin (reviewed in Tanaka et al., 2006). For example, during lateral root initiation, auxin is transported to the pericycle founder cells by a coordinated action of both influx and efflux membrane proteins. Upon this accumulation of auxin in specific lateral root founder cells, a signal transduction cascade is activated that leads to the first formative asymmetric cell divisions (Péret et al., 2009). Consequently, mutants with a disrupted auxin signaling cascade or with a defect in the auxin transport system have a disturbed lateral root phenotype (Fukaki et al., 2002; Benkova et al., 2003).

As the plant rhizosphere harbors many different microorganisms, plant roots are also continuously subjected to a plethora of biotic stresses. Many of them also alter root architecture; for example, plant growth–promoting rhizobacteria and mycorrhizal fungi can stimulate root growth or root branching (Gianinazzi-Pearson, 1996; Persello-Cartieaux et al., 2003). More intriguingly, some microorganisms are able to induce the formation of new root structures. Two such structures that are widespread in nature are the nodules on legume roots induced by symbiotic nitrogen-fixing bacteria and nematode feeding sites (NFSs) formed by plant-parasitic nematodes (Figure 1). Similar to lateral root formation, both structures are initiated in the differentiated root zone and involve a reactivation of the cell cycle and a subsequent redifferentiation process. Since the discovery of high levels of auxin in nodules (Thimann, 1936) and galls (Balasubramanian and Rangaswami, 1962), it had been speculated that auxin plays a role in their organogenesis (Figure 1). However limited information is available regarding which molecular elements of their hosts’ auxin transport machinery are targeted. Since correct localization, transport, and accumulation of auxin are important during the formation of plant organs (Benjamins and Scheres, 2008), recent insights into the molecular regulation of polar auxin transport are opening new avenues for understanding the dynamic auxin distributions occurring during NFS and nodule formation.

This review will highlight auxin transport mechanisms and discuss how plant root-colonizing symbionts and parasites have evolved to manipulate these mechanisms for their own purposes. We will focus on two well-studied interactions (i.e., the legume-rhizobia endosymbiosis and the parasitic plant–nematode interaction).
Regulation of Auxin Transport during Plant Development

The plant hormone auxin is synthesized by all higher plants. The most abundant form of auxin is indole-3-acetic acid (IAA). IAA is synthesized mainly in young shoot tissues and transported from there to other parts of the plant, although other tissues and even specific cell layers also have the capacity to synthesize auxin (Ljung et al., 2001; Ljung et al., 2005; Petersson et al., 2009). Auxin is transported from the shoot to the root tip through the vascular tissue (Aloni, 2004) and from the root tip to the elongation zone through epidermal cells (Mitchell and Davies, 1975). At least two mechanisms of auxin transport have been demonstrated in plants, one via the phloem from source to sink tissues and one by active polar auxin transport across membranes via auxin transport proteins.

Intuitively, the speed and distance often required to move auxin from the shoot meristem to the root tip, particularly in larger plants, could only realistically be achieved in a passive way via the vascular system (Baker, 2000). Nevertheless, the majority of auxin transport research to date has focused on polar auxin transport because chemical inhibitors and Arabidopsis thaliana mutants have been identified that disrupt this process, causing pleiotropic developmental defects during embryogenesis (Friml et al., 2002, 2003), root patterning (Blilou et al., 2005), and lateral root formation (Benkova et al., 2003). The PIN family can be subdivided into full-length and short endomembrane proteins (Mravec et al., 2009). The full-length PIN proteins, especially PIN1, are continuously cycled between the plasma membrane and endosomal compartments (Geldner et al., 2001). In this way, their polarity can be modulated rapidly in response to developmental or external cues, thus redirecting auxin wherever needed. By contrast, PGP auxin transporters are localized more symmetrically and function mainly in nonpolar auxin efflux (Mravec et al., 2008), while a reversible active transport mechanism mediated by PGP4 can contribute to auxin uptake in some cells (Santelia et al., 2005; Terasaka et al., 2005; Yang and Murphy, 2009). PGP1 and PGP19 both interact with the immunophilin-like protein TWISTED DWARF1 (TWD1), and these complexes seem to be required for proper plant development (Geisler et al., 2003, 2005). It is speculated from studies with mammalian MDR transporters that PGP s act as heterodimers (Ramaen et al., 2005). Although not yet demonstrated in plants, active polar auxin transport is orchestrated by a complex interaction of specific influx and efflux carriers. The Arabidopsis genome encodes four putative auxin influx carriers: AUXIN RESISTANT1 (AUX1) and the LIKE AUX1 (LAX1), LAX2, and LAX3 genes (Parry et al., 2001). The AUX1 and LAX3 proteins are plasma membrane localized and have been shown to actively transport auxin into cells (Bennett et al., 1996; Swarup et al., 2004, 2008; Yang et al., 2006). Upon auxin uptake, which also can occur by diffusion, auxin is deprotonated (IAA−) and trapped in the cell. Genetic approaches in Arabidopsis have identified two groups of proteins that are involved in auxin export from cells: PIN-FORMED (PIN) and MULTIDRUG RESISTANCE/P-GLYkoprotein (MDR/PGP) proteins. Each of these families of plasma membrane–localized proteins represents a distinct auxin transport mechanism and have been shown to perform cellular auxin efflux in both plant and heterologous systems (Petrasek et al., 2006; Mravec et al., 2008). The PIN family can be subdivided into full-length and short endomembrane proteins (Mravec et al., 2009). The full-length PINs 1/2/3/4 and 7 have been studied extensively, and it has been clearly demonstrated that they give directionality to auxin transport by means of their asymmetric subcellular localization patterns (Wisniewska et al., 2006). PIN proteins are the key players in establishing auxin maxima, which are essential for organogenesis and meristematic activity (Benková et al., 2003; Bilou et al., 2005). The full-length PIN proteins, especially PIN1, are continuously cycled between the plasma membrane and endosomal compartments (Geldner et al., 2001). In this way, their polarity can be modulated rapidly in response to developmental or external cues, thus redirecting auxin wherever needed. By contrast, PGP auxin transporters are localized more symmetrically and function mainly in nonpolar auxin efflux (Mravec et al., 2008), while a reversible active transport mechanism mediated by PGP4 can contribute to auxin uptake in some cells (Santelia et al., 2005; Terasaka et al., 2005; Yang and Murphy, 2009). PGP1 and PGP19 both interact with the immunophilin-like protein TWISTED DWARF1 (TWD1), and these complexes seem to be required for proper plant development (Geisler et al., 2003, 2005). It is speculated from studies with mammalian MDR transporters that PGP s act as heterodimers (Ramaen et al., 2005). Although not yet demonstrated in plants,
PGPs also bind the auxin transport inhibitor naphthylphthalamic acid, and their mutants as well as twd1 show severe auxin transport-mediated and organogenesis defects (Geisler et al., 2003; Blakeslee et al., 2007). Direct experimental evidence suggests that PGP5s function in restricting auxin to the primary transport streams and in preventing reuptake/reflux of auxin, especially in small cells where PGP5s are abundant and auxin concentrations are high (Bandopadhyay et al., 2007; Blakeslee et al., 2007). Models of how both PIN and PGP proteins work together in transporting auxin have recently been proposed by Blakeslee et al. (2007), Mravec et al. (2008), Titapiwatanakun et al. (2009), and Yang and Murphy (2009). In cells where PINs do not colocalize with PGP5s, the PGP5s regulate the effective cellular auxin concentration available for PIN-mediated transport. By contrast, a direct interaction between PINs and PGP5s, which takes place at polar membrane domains, contributes to the specificity and modulation of auxin efflux rate.

Besides these transport proteins, flavonoids have been postulated as endogenous regulators of auxin transport (Murphy et al., 2000; Brown et al., 2001). Flavonoids are phenylpropanoid metabolites of higher plants with a range of functions (Winkel-Shirley, 2001). Flavonoid-deficient plants show higher rates of auxin transport, whereas mutants overaccumulating flavonols show decreased auxin transport rates (Murphy et al., 2000; Brown et al., 2001; Peer et al., 2004; Wasson et al., 2006). Specific flavonoids, like quercetin and kaempferol inhibit auxin transport by competing with synthetic auxin transport inhibitors (including naphthylphthalamic acid and 2,3,5-triodobenzoic acid) for plasma membrane and microosomal binding sites (Stenlid, 1976; Jacobs and Rubery, 1988; Bemascon, 1996). It has been shown that a lack of flavonoids in Arabidopsis mutants alters the expression and localization of certain PIN proteins (Peer et al., 2004), while a flavonoid treatment to pin2 mutants partially rescues their root gravitropic phenotype by redirecting PIN-mediated auxin fluxes (Santelia et al., 2008). However, it remains to be established whether PIN5 protein localization is directly altered by flavonoids, perhaps by acting on vesicular trafficking proteins (Peer and Murphy, 2007), or whether the altered PIN protein movement is a consequence of flavonoid-mediated alterations in auxin transport (Peer et al., 2004).

Flavonoids might be expected to influence PGP-mediated auxin transport. For example, it has been demonstrated that the flavonols quercetin and kaempferol disturb the binding between PGP5s and their activator TWISTED DWARF (Bailly et al., 2008). Flavonoids also are known to accumulate in plant tissues in response to a variety of bacteria, fungi, and nematodes (Harrison and Dixon, 1993; Dakora and Phillips, 1996; Stafford, 1997; Mathiesius et al., 1998b; Hutangura et al., 1999; Jones et al., 2007). Therefore, although their function in regulating auxin transport is still questionable, flavonoids could be targets for the regulation of auxin transport by microbes. Evidence for this hypothesis is discussed below.

The Legume–Rhizobium Interaction

The establishment of nitrogen-fixing nodules is probably the most studied symbiotic interaction between soil bacteria and plants. Many legumes enter into this symbiosis with nitrogen fixing bacteria, collectively called rhizobia. The interaction is initiated by a dual recognition. The host roots exude flavonoids or betaines, which stimulate the synthesis of so-called Nod factors (lipochitin oligosaccharides) by the bacterial partners. These Nod factors are subsequently perceived by the host plant, which in turn triggers the organogenesis of the root nodule, required for the accommodation of rhizobia (Oldroyd et al., 2009). Inside the nodule, rhizobia convert atmospheric nitrogen into ammonia, which is exported to the plant in exchange for carbohydrates.

Nodule organogenesis and nodule structure in legumes are very diverse. Indeterminate nodules, which are formed in many temperate legumes like pea (Pisum sativum) and alfalfa (Medicago sativa), are initiated in the pericycle and inner cortical cells adjacent to the xylem pole. The cell divisions in the cortical cells will form the nodule primordia, whereas divisions in the pericycle are suggested to lead the vascularization of the newly developed organ (de Billy et al., 2001). These cell divisions result in a nodule with a persistent meristem (Figure 1). Determinate nodules, on the other hand, are typically formed on tropical legumes like beans (Phaseolus vulgaris) and soybeans (Glycine max) and originate from outer cortical cell divisions and enlargements. These cells subsequently fuse with a group of dividing pericycle cells. Although many more nodule types with different organogenesis pathways exist (e.g., nodule development based on modification of existing lateral or adventitious roots [Hirsch and LaRue, 1997]), this review will focus on determinate and indeterminate nodules.

Nodule Organogenesis Is Characterized by Changes in Auxin Response

Auxin is important in cell cycle regulation and plant organogenesis and therefore is likely to be associated with morphological changes caused by microbes in plants. Indeed, it was shown that in indeterminate nodules formed on white clover (Trifolium repens) and Medicago truncatula (Mathiesius et al., 1998a; Hoo et al., 2006; van Noorden et al., 2007), increased auxin response (measured as increased staining from auxin responsive elements, such as GH3 or DR5 fused to β-glucuronidase [GUS marker]) occurred in the early dividing cells of the nodule primordium (Figure 1) and in the pericycle and inner cortical cells. In determinate nodules of the legume Lotus japonicus, an enhanced auxin response was observed in the outer cortical cells (Pacios-Bras et al., 2003). Moreover, a proteomic study in M. truncatula showed that there is a high overlap (~90%) between protein changes occurring during early nodulation and in roots treated with auxin (van Noorden et al., 2007). This suggests that increased auxin response and/or concentration in the root may mediate a substantial number of root responses to rhizobia.

The enhanced auxin response could result from stimulated auxin biosynthesis or reduced auxin breakdown in the host at the site of infection, enhanced auxin sensitivity, or effects on auxin transport. Alternatively, auxin accumulating in the nodule primordium could be rhizobia derived. Rhizobia have the ability to synthesize auxin, and studies with Bradyrhizobium mutants deficient in IAA synthesis have shown that nodule numbers are reduced in soybean by a lack of rhizobial auxin (IAA) synthesis
Moreover, increased nodule numbers were observed in *M. truncatula* plants inoculated with a *Sinorhizobium meliloti* strain that overproduces IAA (Pié et al., 2007). However, auxin produced by rhizobia is thought not to be necessary for the initiation events of the nodule, as in many legumes Nod factors alone can induce the early stages of nodule development, such as cortical cell divisions and auxin transport inhibition events leading to the initiation of indeterminate nodules (Mathesius et al., 1998a; Boot et al., 1999).

**Rhizobia Alter Host Auxin Transport**

The organogenesis of lateral roots and that of nodules share several aspects; one of them is that both lateral roots and nodules are initiated in front of a xylem pole upon an accumulation of auxin. In *Arabidopsis*, auxin gradients needed for lateral root initiation rely on a functional auxin transport system (Benková et al., 2003), which might suggest that nodule development depends on the auxin transport mechanisms of their hosts. This suggestion is supported by the observation that external application of synthetic auxin transport inhibitors to roots inhibits nodulation (van Noorden et al., 2006), although in some legumes, long-term application of auxin transport inhibitors can induce nodule-like structures (Hirsch et al., 1989; Wu et al., 1996). It is likely that temporary inhibition of polar auxin transport at the infection site is a prerequisite for indeterminate nodule formation, as rhizobia and purified Nod factors inhibit the auxin response in white clover roots expressing an auxin-responsive GUS reporter below the site of inoculation, similar to the action of synthetic auxin transport inhibitors (Mathesius et al., 1998a). In addition, transport of radiolabeled auxin was reduced in indeterminate nodules of *vetch (Vicia sativa)* and *M. truncatula* following inoculation with rhizobia (Boot et al., 1999; van Noorden et al., 2006; Wasson et al., 2006). However, in contrast with the indeterminate nodules, no auxin transport inhibition was detected in the determinate nodules of *L. japonicus* during nodule initiation (Pacios-Bras et al., 2003), indicating a difference in auxin requirement for nodule initiation between indeterminate and determinate nodules.

Analysis of the molecular components of the auxin transport machinery during nodule initiation has been limited, mainly because the genomes of both *M. truncatula* and *L. japonicus* (the model plants for indeterminate and determinate nodulation, respectively) to date have not been completely sequenced. In *M. truncatula* and *L. japonicus*, several PIN genes (10 and 2, respectively) have been identified based on sequence similarity with *Arabidopsis* (Schnabel and Frugoli, 2004). A reporter study demonstrated that Mt-PIN2 has a similar expression pattern in the root as At-PIN2 in *Arabidopsis* and that the expression pattern changes of Mt-PIN2 during early nodulation has a strong resemblance to the pattern of expression during lateral root initiation (Huo et al., 2006). Silencing several of the PIN genes reduced nodulation in *M. truncatula* (Huo et al., 2006), supporting the hypothesis that a functional auxin transport system is required for nodulation.

In addition to the auxin exporters, five AUX1-like (LAX) genes were identified in *M. truncatula*, and at least one LAX gene (but possibly four) was found in *L. japonicus* (de Billy et al., 2001; Schnabel and Frugoli, 2004). In situ hybridization studies, using probes targeting three Mt-LAX genes simultaneously, demonstrated that Mt-LAX is highly expressed in lateral root and nodule primordia. At later stages, the genes are expressed in peripheral tissues of a nodule and central tissues of lateral roots. Based on these experiments, the researchers suggested that import-mediated auxin localization is not only needed for primordia initiation but also for vasculature differentiation (de Billy et al., 2001).

In *Casuarina glauca*, which forms a nitrogen-fixing endosymbiosis with the actinomycete *Frankia* sp, the ortholog of *Arabidopsis* AUX1 was induced in root cells colonized by the symbiont (Péret et al., 2007). The authors suggested that auxin synthesized by *Frankia* sp is transported into colonized host cells via AUX1 and that this is a necessary step in plant cell infection. Although AUX1 was not directly involved during initiation and development of a nodule, this was the first report of an auxin import activity linked to plant cell infection by a soil microorganism (Péret et al., 2007).

How auxin importers and exporters are regulated during nodulation is still unknown. One of the possibilities is that PIN expression might be regulated by ethylene, as the expression of both Mt-PIN1 and Mt-PIN2 is upregulated in the ethylene insensitive *M. truncatula sickle* mutant within 24 h at the site of nodule initiation (Prayitno et al., 2006). Another possibility is the involvement of flavonoids. Flavonoids may be considered as regulators of auxin transport (see above) and specifically accumulate at the site of *Rhizobium* infection (Mathesius et al., 1998b). In white clover expressing an auxin reporter GUS construct, local treatment of roots with the flavonoids kaempferol and quercetin resulted in a similar reduction of the auxin response as seen in *Rhizobium*-inoculated roots (Mathesius et al., 1998a). Moreover, in flavonoid-deficient *M. truncatula* roots, rhizobia were unable to inhibit the host’s auxin transport, and consequently these roots were not capable of developing nodules (Wasson et al., 2006). Kaempferol is a likely candidate to regulate auxin transport during nodule initiation, as it was shown that biosynthesis of kaempferol was stimulated by rhizobia in *M. truncatula* and that exogenously applied kaempferol was able to inhibit auxin transport in flavonoid-deficient roots and restore nodulation (Zhang et al., 2009).

In contrast with indeterminate nodule initiation, (iso)flavonoids were shown not to be required for determinate nodule development in soybean (Subramanian et al., 2006). Auxin transport inhibition appears to be specific for indeterminate nodulation, possibly reflecting the different requirements for cell division in either inner or outer cortical cells in the two types of nodules (Mathesius, 2008). These data clearly indicate the importance of flavonoids in the regulation of auxin transport during nodulation. However, whether and how PIN, LAX, or PGP proteins are regulated by flavonoids during nodulation or other plant organ development has not been established.

In addition to the importance of local auxin transport at the nodule initiation site, rhizobia also alter shoot-to-root auxin transport via systemic signals. Inoculation of roots with rhizobia triggers autoregulation, a systemic control that limits the number of nodules on a root system (Caetano-Anolles and Gresshoff, 1991). Autoregulation mutants are defective in a leucine-rich receptor like kinase and supernodulate (Stacey et al., 2006). *M. truncatula* autoregulation mutant super numeric nodules...
(sunn) is characterized by increased auxin transport from the shoot to the root (van Noorden et al., 2006). Whereas inoculation of wild-type plants with rhizobia inhibits shoot-to-root auxin transport, auxin transport in sunn remains unaffected, suggesting that inhibition of systemic auxin transport is part of the autoregulation control (van Noorden et al., 2006). So far, none of the signals regulating systemic control of auxin transport by rhizobia have been identified.

**Nematode Infection**

In contrast with the symbiotic relationship between legumes and rhizobia, plant-parasitic nematodes are harmful pathogens, and host plants severely suffer from their infection. Sedentary nematodes (as opposed to migratory species) establish an intimate relation with their hosts and in that manner resemble endosymbiotic rhizobia. Within the sedentary endoparasitic nematodes, two major groups can be distinguished based on their infection process: the root-knot and the cyst nematodes (Figure 1). Cyst nematodes select a single cell (a pericycle or a procambial cell near the primary xylem pole in Arabidopsis), while root-knot nematodes pick five to seven neighboring vascular cells (Golinowski et al., 1996; Bleve-Zacheo and Melillo, 1997). It is now generally accepted that nematodes use their stylet secretions to orchestrate elaborate root cell modifications, finally resulting in the establishment of specialized feeding cells (Davis et al., 2004). The initial cell chosen by cyst nematodes undergoes a redifferentiation process during which the nucleus enlarges by endoreduplication and cell walls are partially dissolved (Golinowski et al., 1997). Gradually, the neighboring cells fuse and a feeding site or syncytium takes shape (Figure 1). During nematode development, the syncytium expands by integrating more adjacent cells. A fully developed syncytium may ultimately incorporate several hundred cells. In contrast with this, the cells selected by root-knot nematodes are stimulated to go through synchronous repeated nuclear divisions without cytokinesis, while the nuclei additionally undergo extensive endoreduplication (Bleve-Zacheo and Melillo, 1997). The resulting hypertrophied mature giant cells may contain >100 polyploid nuclei (Wiggers et al., 1990). To accommodate the growing giant cells, surrounding pericycle and cortical cells also enlarge and divide. As a result, the infection is accompanied by pronounced and characteristic galling of the surrounding tissue (root-knot) (Figure 1).

**Nematode Infection Requires Changes in the Host’s Auxin Response and Transport**

The impressive transformation of the plant cell infected by sedentary endoparasitic nematodes goes along with a plethora of physiological and molecular changes (Jammes et al., 2005; Ithal et al., 2007; Gheyseg and Mitchum, 2009). Similar to nodule initiation, an enhanced auxin response has been visualized at the infection sites of both cyst and root-knot nematodes, whereas auxin signaling mutants have shown significantly lower nematode infection (Hutangura et al., 1999; Gouvea et al., 2000; Karczmarek et al., 2004; Grunewald et al., 2008). This suggests that auxin might trigger the initiation of both giant cells and syncytia and that auxin-dependent programs are needed for NFS establishment.

Although the involvement of nematode-derived auxin cannot be excluded at this time, several studies into plant–parasitic nematode interactions argue for the involvement of the host’s polar auxin transport machinery (Figure 2). For example, the expression of the AUX1 auxin importer is stimulated in young NFS (Mazarei et al., 2003), implying that nematodes actively enhance the transport of auxin in the chosen cells. However, to date, functional analyses using aux and lax mutants have not been performed. The expression of the efflux transporter PIN1 is downregulated in the initial syncytial cell, preventing the drain of auxin and thus assisting AUX1 in accumulating auxin in these cells (Grunewald et al., 2009). Interestingly, auxin accumulation in young NFS is only transient, and between 2 and 5 d after the initial infection the auxin response shifts to the neighboring cells (Hutangura et al., 1999; Karczmarek et al., 2004; Grunewald et al., 2008). This observation argues again for the involvement of auxin transport and resembles the auxin response that occurs during formation of other plant organs. For example, during Arabidopsis embryogenesis, auxin accumulates in the proembryo until the early globular stage (Friml et al., 2003). Subsequently, PIN transporters guide auxin to the hypophysis where it activates developmental programs leading to the formation of the root meristem.

During the plant–nematode interaction, auxin feasibly might reprogram the neighboring cells for integration into the developing syncytium or to activate cell division needed to keep up with the growing giant cells. Indeed, when lateral auxin transport was inhibited chemically or genetically, radial expansion of the syncytial cell was inhibited, and small malformed cysts were produced (Gouvea et al., 2000; Grunewald et al., 2009). Intriguingly, nematodes can influence the polar localization of the PIN proteins, resulting in transport of auxin to the adjacent tissues, as indicated by the redirection of basal localized PIN3 to the lateral plasma membranes (Figure 2) (Grunewald et al., 2009). The question is how nematodes are able to achieve this.

Polarity shifts of PIN proteins are a common feature during important plant developmental processes, and it seems that nematodes have evolved to manipulate this. The PIN polarity switch is highly similar to that during lateral root initiation. At the earliest stage of lateral root initiation, PIN1 is detected exclusively on the anticlinal sides of the short pericycle initial cells (Benková et al., 2003; Kleine-Vehn et al., 2008), but between stages I and III, PIN1 localization undergoes a polarity switch from the anticlinal to the outer periclinal cell side that represents the new basal (lateral root apex–facing) side. Pharmacological and genetic experiments revealed that the ARF-GEF protein GNOM is required for the switches of PIN polarity to the basal cell side (Benková et al., 2008). GNOM is a regulator of PIN trafficking from the endosomes back to the plasma membrane, as shown by its sensitivity to the endosomal trafficking inhibitor Brefeldin A. GNOM seems to be crucial for basal polar targeting because the apical plasma membrane localization of PIN proteins and AUX1 is not strongly affected when GNOM function is inhibited (Kleine-Vehn et al., 2008). Similarly, during Arabidopsis embryogenesis, auxin accumulates in the hypophysis by means of a GNOM-dependent PIN polarity switch (Friml et al., 2003). Until now, no lateral localization...
of PINs has been reported, suggesting that the nematodes’ stylet secretions lead to cell identity changes due to which the lateral plasma membrane becomes the new (root epidermis–facing) basal side. Consequently, basal PIN polarity is redirected by ARF-GEF transcytosis mechanisms. Alternatively, nematodes could alter expression levels of the protein phosphatase 2A (PP2A) and/or the Ser/Thr protein kinase PID since several findings suggest that the PIN polarity signals are related to the phosphorylation sites found in the PIN sequences (Friml et al., 2004; Michniewicz et al., 2007). PID has been shown to directly phosphorylate the hydrophilic loop of PIN proteins in vivo and in vitro, while PP2A phosphatase antagonizes this action (Michniewicz et al., 2007). High levels of PIN phosphorylation, as achieved by overexpression of PID (Benjamins et al., 2001; Friml et al., 2004) or inhibition of PP2A (Michniewicz et al., 2007), lead to a preferential apical PIN targeting. Consequently, the characteristic turnover in auxin transport direction during the globular embryo stage does not occur, resulting in a misspecification of the hypophysis and in rootless seedlings (Friml et al., 2004). Low phosphorylation levels in the pid mutants result in a preferential basal PIN targeting (Friml et al., 2004; Tremel et al., 2005), yielding pin1-like inflorescences (Benjamins et al., 2001). As described in these studies, many of the mutants affected in PIN polarization are severely impaired in their development, including root growth. Therefore, more innovative approaches (e.g., inducible loss-of-function strategies) must be designed to elucidate the function of these genes during nematode infection.

How Is Laterally Transported Auxin Localized?

During the establishment of the NFS, both PIN and AUX-mediated mechanisms work together to transport and localize auxin, respectively. A good candidate to localize the radially transported auxin is LAX3 due to its similar function in lateral root outgrowth. During lateral root initiation, auxin induces the expression of LAX3 in cortical and epidermal cells directly overlying new primordia (Swarup et al., 2008). Recently, it was demonstrated that LAX3-dependent auxin signaling induces a number of cell wall–remodeling enzymes, which are likely to promote cell separation to allow emergence of the developing lateral root primordia (Swarup et al., 2008). In a similar manner, giant cells must push away several layers of host tissue to grow radially, and syncytia must reprogram neighboring cells to integrate them. Indeed, endoglucanases, expansins, and several other host cell wall modifying enzymes are active in the NFS overlying cells (Goellner et al., 2001; Vercauteren et al., 2002; Wieczorek et al., 2008; Gheysen and Mitchum, 2009). This not only suggests that LAX3 could be involved in the auxin accumulation in neighboring cells but also that the auxin-dependent activation mechanisms of cell wall–modifying enzymes could be identical in NFS and in lateral root outgrowth.

Are Flavonoids Involved in Nematode Infection?

There is increasing interest in the possible effects of flavonoids as modulators of auxin transport during plant–microbe interactions, and, as mentioned above, flavonoids play crucial roles during the initiation of indeterminate nodules (Wasson et al., 2006; Zhang et al., 2009). Because of the close resemblance between nodules and NFS, it has been postulated that flavonoids might also have a role during nematode infection (Hutangura et al., 1999). Increased promoter activity of CHALCONE SYNTHASE, encoding the first enzyme in the biosynthesis of flavonoids, has been demonstrated in Meloidogyne javanica–induced
galls in clover roots (Hutangura et al., 1999). Similar to the accumulation of flavonoids in nodules and lateral root primordia, root galls are characterized by the accumulation of flavonoids in cortical and vascular cells (Hutangura et al., 1999; Wasson et al., 2009). However, flavonoid-deficient Arabidopsis mutants were still able to form feeding sites when infected with cyst nematodes (Jones et al., 2007) or with root-knot nematodes (Wyuts et al., 2006). Furthermore, flavonoid deficiency in M. truncatula did not prevent the formation of galls or giant cells, although the number of divided pericycle cells around the galls was reduced (Wasson et al., 2009). These studies indicate that flavonoids likely do not mediate the required changes in auxin transport needed for NFS initiation and development.

CONCLUSIONS

Symbiotic and parasitic interactions between species and especially their molecular crosstalk, are fascinating features in nature. It is even more intriguing that organisms such as rhizobia and nematodes can take control of their interacting partners and are able to induce new structures in their hosts’ root systems. Prior to the establishment of nodules, NFS, and lateral roots induced by such organisms, an accumulation of auxin can be observed. Auxin might have dual roles in guiding the development of these root structures, first as a morphogen specifying the site of organ formation (Benková et al., 2009) and second as a regulator of the plant cell cycle (Himanen et al., 2002). Therefore, it is not surprising that auxin is a target for microorganisms that manipulate plant development and consequently that plants have evolved mechanisms to repress auxin signaling during infection as a defense strategy (Wang et al., 2007; Kazan and Manners, 2009). However, the mechanisms by which root symbionts and parasites take over endogenous programs to localize and redirect auxin transport are not well understood.

Although the new initiation and subsequent redifferentiation of pericycle and cortical cells is similar for NFS and nodule development, and involves the accumulation of auxin at the early stages of organogenesis, our current knowledge suggests that the mechanism of auxin accumulation varies. Whereas nema-
tode parasitism in plants has been suggested to have evolved several times, possibly 200 to 300 million years ago (Baldwin et al., 2004), the Rhizobium-legume symbiosis occurred more recently, at ~60 million years ago (Sprent, 2007). NFSs typically develop after nematodes secrete a complex mixture of proteins into plant cells, while nodules result from the perception of a Nod factor molecule by a host-specific receptor that triggers a signal transduction cascade. In both interactions, the microorganism causes rearrangements of PIN and AUX/LAX transporters, but only nodules appear to require the action of flavonoids as putative auxin transport regulators. How are PIN and AUX/LAX transporters regulated in the case of NFS development? One possibility is that proteins or peptides injected into the plant host directly interfere with the transcription of auxin transport regulators (e.g., PID and PP2A). A recent report identified >400 proteins of the secretome of a root-knot nematode, including several putative transcriptional regulators, although their targets remain elusive (Bellafiore et al., 2008). Alternatively, auxin itself can alter its own transport by relocating PIN proteins (Sauer et al., 2006), so any alterations of auxin concentration could also affect changes in auxin transporters.

A possible focus for future studies is nematode signaling molecules that might alter auxin transport proteins. In addition, it will be important to determine how the Nod factor signal transduction cascade leads to changes in flavonoid accumulation at specific sites to alter auxin transport and accumulation. Another area of future study lies in the elucidation of the roles of PGP proteins in the control of auxin transport in symbiotic and parasitic interactions. While interesting in their own right, both rhizobia and nematodes are excellent tools to study the regulation of auxin transport in plant development. In both cases, a new plant organ can be triggered de novo by external signals. Future studies might take advantage of these inducible plant organogenesis programs to gain new insights into our understanding of how auxin transport regulation determines plant development.

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Manipulation of Auxin Transport in Plant Roots during *Rhizobium* Symbiosis and Nematode Parasitism

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