J Plant Growth Regul (2000) 19:385–396
DOI: 10.1007/s003440000041

J P G R
Journal of Plant Growth Regulation

Maintenance of Asymmetric Cellular Localization of an Auxin Transport Protein through Interaction with the Actin Cytoskeleton

Gloria K. Muday*

Department of Biology, Wake Forest University, Winston-Salem, North Carolina 27109-7325, USA

Abstract

In shoots, polar auxin transport is basipetal (that is, from the shoot apex toward the base) and is driven by the basal localization of the auxin efflux carrier complex. The focus of this article is to summarize the experiments that have examined how the asymmetric distribution of this protein complex is controlled and the significance of this polar distribution. Experimental evidence suggests that asymmetries in the auxin efflux carrier may be established through localized secretion of Golgi vesicles, whereas an attachment of a subunit of the efflux carrier to the actin cytoskeleton may maintain this localization. In addition, the idea that this localization of the efflux carrier may control both the polarity of auxin movement and more globally regulate developmental polarity is explored. Finally, evidence indicating that the gravity vector controls auxin transport polarity is summarized and possible mechanisms for the environmentally induced changes in auxin transport polarity are discussed.

Key words: Auxin transport; Actin cytoskeleton; Polarity; F-actin; Gravity; Embryo development

Introduction

The mechanism by which cells and tissues develop and maintain polarity is a growing area of study. In mammalian systems, a number of proteins have been examined to understand how asymmetric cellular localization is established and maintained. In asymmetric cells, such as epithelial or nerve cells, there are mechanisms both for initially sorting proteins into different membrane domains and for maintaining the localization after initial sorting is complete (Drubin and Nelson 1996; Nelson and Grindstaff 1997). Protein sorting through directed vesicle targeting is critical for establishment of asymmetry (Nelson and Grindstaff 1997), whereas attachment to the actin cytoskeleton, either directly or through large protein complexes, maintains the asymmetric localization (Colledge and Froehner 1998; Froehner 1993).

In plant cells, one of the best-understood examples of a protein with asymmetric cellular localization is the auxin efflux carrier, which controls

Online publication 2 May 2001
Corresponding author; e-mail: muday@wfu.edu

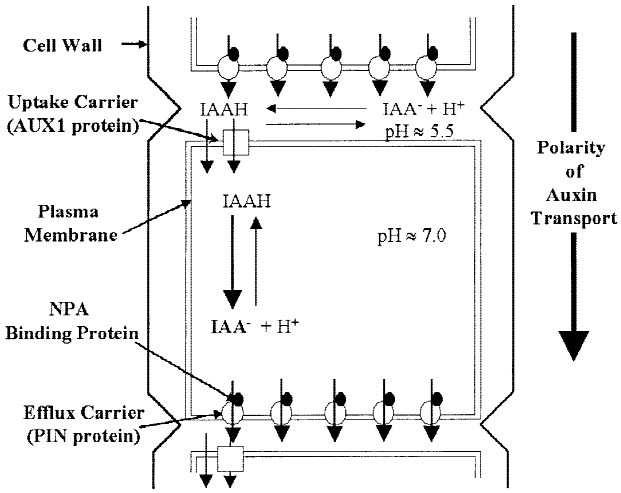

Figure 1. Schematic model of the chemiosmotic hypothesis for polar auxin transport. Protonated IAA in the cell wall space can enter the cell either by diffusion or by an uptake carrier. Once in the more basic cytoplasm, the IAA dissociates and can exit only by the auxin efflux carrier. The names of the protein that may constitute auxin transporters are in parentheses. *Reprinted from Muday (2000) with kind permission from Kluwer Academic Publishers.*

polar auxin transport. Auxins, of which indole-3-acetic acid (IAA) is the predominant naturally occurring hormone, move through plants by a unique polar transport mechanism (reviewed in Goldsmith 1977; Lomax and others 1995). This polar movement of auxin in the shoot is a cell-to-cell movement from the shoot apex toward the base. Polar auxin transport results in an auxin gradient down the length of the plant, with the highest auxin concentrations found in the regions of greatest elongation (Ortuno and others 1990). There are two protein complexes that control auxin movement into and out of cells (Lomax and others 1995). These protein complexes, the auxin uptake carrier, and the auxin efflux carrier, respectively, are shown in Figure 1. IAA can move into cells both passively, because it is hydrophobic when protonated, and through an uptake carrier. The amount and direction of polar auxin transport are thought to be controlled by the auxin efflux carrier. Basal localization of an auxin efflux carrier in cells of the plant stem has also been proposed to determine the polarity of IAA transport (Gälweiler and others, 1998; Jacobs and Gilbert 1983; Rubery and Sheldrake 1974).

This review focuses on the asymmetric localization of the auxin efflux carrier. Evidence will be summarized that indicates that the auxin efflux carrier complex may be directed to one membrane through vesicle targeting and that this protein complex interacts with the actin cytoskeleton to fix this protein in one domain of the plasma membrane. In addition, the idea is explored that this localization of the efflux carrier may control both the polarity of auxin movement and globally regulate developmental polarity. Finally, experimental results are summarized that suggest that environmental stimuli can change the polarity of auxin transport, and the possible roles of the cytoskeleton in mediating these alterations in auxin transport direction are discussed.

Biochemical Characterization of the Auxin Efflux Carrier

A biochemical dissection of the auxin efflux carrier has increased our understanding of the regulation of this protein complex and how its localization to the basal plasma membrane controls the polarity of auxin movement. The efflux carrier complex appears to be composed of at least two polypeptides. One polypeptide is an integral membrane transporter presumably encoded by one of the members of the *PIN* gene family (Palme and Gälweiler 1999). A second polypeptide (the NPA-binding protein) binds synthetic inhibitors of auxin efflux, including naphthylphthalamic acid, and may act as a regulatory polypeptide (Dixon and others 1996; Muday 2000). The existence of a third, rapidly turned over protein that connects these two subunits has also been suggested (Morris and Robinson 1998; Morris and others 1991).

Several members of the *PIN* gene family in *Arabidopsis* have been identified (Gälweiler and others 1998; Müller and others 1998), indicating that there are multiple auxin efflux carriers with distinct expression patterns (Palme and Gälweiler 1999). Four groups published the isolation of the *PIN2* gene, within several months, but using different names. *PIN2* is equivalent to *EIR1*, *AGR1*, and *WAV6* (Chen and others 1998; Luschnig and others 1998; Müller and others 1998; Utsuno and others 1998). Plants with mutations in either *PIN1* or *PIN2* have phenotypes consistent with tissue-specific alterations in auxin transport (Gälweiler and others 1998; Müller and others 1998; Okada and others 1991) and have alterations in auxin transport in the affected tissues (Chen and others 1998; Okada and others 1991; Rashotte and others 2000). *PIN* genes encode proteins with 10 membrane-spanning domains and have similarities to other membrane transport proteins (Chen and others 1999). The protein products of these genes show an asymmetric localization in the plasma membrane that is consistent with con-

trolling the polarity of auxin movement (Gälweiler and others 1998; Müller and others 1998). It has been suggested, therefore, that the *PIN* genes encode one polypeptide of the auxin efflux carrier.

Until the recent identification of the PIN proteins, most studies of the auxin efflux carrier focused on the NPA-binding protein. The activity of this protein can be followed using a binding assay with [^{3}H]-NPA. NPA binds with high affinity to a single class of NPA-binding proteins associated with the zucchini plasma membrane (Muday and others 1993). Therefore, this assay has allowed extensive biochemical characterization of the NPA-binding protein. In addition, an [^{3}H]-azido NPA derivative has been synthesized (Voet and others 1987) and has been used to identify a 23-kDa protein that binds both NPA and IAA (Zettl and others 1992).

Several lines of evidence suggest that the protein that binds inhibitors of auxin efflux, including NPA, is distinct from the *PIN* gene products. Treatments with inhibitors of protein translation or protein processing in the Golgi apparatus prevent the NPA-mediated reductions in auxin transport without altering the amount of NPA-binding activity (Morris and Robinson 1998; Morris and others 1991; Wilkinson and Morris 1994). These results suggest that the NPA-binding and auxin-efflux activities are on separate proteins and support the idea that a third protein may connect them (Morris and others 1991).

In addition, it appears that the NPA-binding protein is peripherally associated with the plasma membrane, and the NPA binding site is on the cytoplasmic face of the membrane (Muday 2000). Treatment of plasma membrane vesicles with concentrated salt solutions released the NPA-binding protein into the supernatant after ultracentrifugation, suggesting that NPA binds to a peripheral protein (Cox and Muday 1994). Furthermore, the NPA-binding protein is still active in pellets obtained after detergent solubilization of plasma membranes followed by ultracentrifugation (Butler and others 1998; Cox and Muday 1994). These pellets should be almost free of lipids, yet most NPA-binding activity was recovered in these pellets, suggesting that the NPA-binding protein does not require a lipophilic environment to be active (Butler and others 1998; Cox and Muday 1994).

Biochemical evidence suggests that NPA-binding activity is localized to the cytoplasmic face of the plasma membrane. Several investigators have examined the protease sensitivity of NPA-binding activity in plasma membranes isolated from zucchini hypocotyls. Treatment of intact right-side-out vesicles with protease does not lead to loss of NPA-binding activity (Bernasconi and others 1996; Dixon and others 1996), whereas disruption of membranes by detergent, followed by protease treatment, results in a total loss of NPA-binding activity (Bernasconi and others 1996). Furthermore, plasma membrane vesicles have been subjected to several different treatments that should convert them to an inside-out orientation (Bernasconi and others 1996; Dixon and others 1996; Hertel and others 1983), although the effectiveness of these treatments was only verified by analysis of marker enzymes in one case (Dixon and others 1996). In two of these reports, inside-out vesicles had twofold to fourfold increases in NPA-binding activity and fourfold to fivefold increases in protease sensitivity of NPA-binding activity compared with right-side-out vesicles (Dixon and others 1996; Hertel and others 1983). Therefore, the NPA-binding site appears to be localized to the cytoplasmic face of the membrane and poised for interaction with the cytoskeleton.

Evidence for NPA-Binding Protein Interaction with Actin

One of the hallmarks of proteins that interact with the cytoskeleton is their retention in the pellet after ultracentrifugation of detergent-extracted membranes (Carraway 1992). So, the first experiments designed to ask whether the NPA-binding protein interacts with the cytoskeleton involved examination of the behavior of this protein during detergent extractions. Although several reports in the literature indicate that some NPA-binding activity can be released from the membrane by detergent treatment, all of these procedures resulted in very low yields of soluble NPA-binding activity (Bernasconi and others 1996; Cox and Muday 1994; Jacobs and Gilbert 1983; Sussman and Gardner 1980). In two of these reports, the amount of NPA-binding activity in the detergent-insoluble pellet was quantified and, in both cases, most of the activity was in the detergent-insoluble pellet (Cox and Muday 1994; Sussman and Gardner 1980).

When either purified zucchini plasma membranes or fresh and relatively crude extracts of zucchini are treated with Triton X-100, almost all of the NPA-binding activity and the cytoskeletal proteins, actin and tubulin, were in the pellet after ultracentrifugation (Butler and others 1998). The abundance of NPA-binding activity and actin in the detergent-insoluble pellet is decreased by treatments with drugs or agents that fragment the actin filaments (F-actin). There is a twofold decrease by addition of cytochalasin D or a fourfold to 10-fold decrease on

treatment with the buffer Tris, depending on the Tris concentration (Butler and others 1998; Cox and Muday 1994). In addition, treatment with phalloidin, an F-actin stabilizing drug, increased both the amount of pelletable actin and NPA-binding activity by greater than threefold. In contrast, taxol treatment stabilized microtubules and resulted in an increase in pelletable tubulin after detergent solubilization but caused no detectable change in pelletable NPA-binding activity (Butler and others 1998). These partitioning experiments indicate that treatments that stabilize actin increase the NPA-binding activity recovered in the detergent-insoluble cytoskeleton fractions, whereas treatments that destabilize the actin cytoskeleton decrease the amount of NPA-binding activity recovered in these samples. Therefore, these results are consistent with an actin interaction of the NPA-binding protein, but more direct demonstration was necessary to strengthen these results.

It was also necessary to ask whether the NPA-binding protein interacts directly with purified actin filaments. F-actin affinity chromatography is one approach to demonstrate this interaction. First, a procedure for purification of zucchini hypocotyl actin using its ability to bind the enzyme DNase I was developed and used to obtain purified F-actin (Hu and others 2000). A variety of methods were used to demonstrate that the actin was native, including the demonstration of the ability of actin monomers (G-actin) to bind profilin, to bind and inhibit DNase I activity, and to form filaments as judged by ultracentifugation, native gel electrophoresis, and electron microscopy (Hu and others 2000; Muday 2000).

Purified and functional zucchini hypocotyl actin was then used to prepare both G- and F-actin columns. BSA was used to create a third affinity matrix to test for nonspecific protein interactions (Hu and others 2000). The selectivity of the F-actin column was demonstrated by examination of binding of vertebrate α-actinin to the F-actin column. Purified α-actinin was shown to bind tightly to the F-actin but weakly to the G-actin column (Hu and others 2000).

To test for the ability of the NPA-binding protein to interact with actin filaments, Tris and detergent-solubilized plasma membranes, enriched in actin and actin-binding proteins, were used as the starting sample for chromatography on the actin columns. NPA-binding activity was retained by the F-actin column and reproducibly eluted with high salt concentrations. In five separate experiments, NPA-binding activity was localized to one or two fractions eluted from the F-actin column and was significantly greater than the activity eluted from a BSA column or an F-actin column to which no solubilized proteins were applied (Hu and others 2000). The selective elution of NPA-binding activity from the F-actin column is the strongest evidence to date indicating the association of the NPA-binding protein with the actin cytoskeleton.

Drugs that Fragment the Actin Cytoskeleton Reduce Polar Auxin Transport

The interaction of the NPA-binding protein with the actin cytoskeleton may be necessary for either movement of auxin across the membrane or for the polar localization of the efflux carrier complex. If either of these hypotheses is correct, disruption of the actin cytoskeleton would be predicted to reduce polar auxin transport.

Treatments of either corn coleoptiles (Cande and others 1973) or zucchini hypocotyls (Butler and others 1998) with cytochalasins have been shown to reduce auxin transport. The effect of cytochalasin D on auxin transport was measured in zucchini hypocotyls (Butler and others 1998). The goal of this experiment was to determine whether basipetal auxin transport was reduced by this treatment without affecting acropetal diffusion of IAA or diffusion of the weak acid control, benzoic acid. Zucchini hypocotyl segments treated with and without cytochalasin D are simultaneously loaded with [^{3}H]-IAA and [^{14}C]-benzoic acid, and the amount of radioactivity moving in either direction is quantified. When zucchini hypocotyls are treated with cytochalasin D, there is a statistically significant reduction (as judged by Student's *t* test) in basipetal auxin transport, as shown in Table 1 (data from Butler and others 1998). The average and standard error of 10 separate experiments, each with six replicates, is reported. This 1.4-fold reduction in transport is not at the level of diffusion, because there are no changes in the amount of either basipetal benzoic acid movement or acropetal auxin transport. Because this assay measures passive diffusion from the segment, as well as polar transport, each measurement contains some background diffusion. The level of background diffusion can be assessed by examining the percentage of either acropetal auxin movement or benzoic acid diffusion. Basipetal IAA transport can be normalized by subtracting the amount of diffusion (calculated by averaging percentage of BA movement in either di-

Table 1. Cytochalasin D Reduces Polar Auxin Transport

	%Transport[a]		
	−Cytochalasin D	+Cytochalasin D	*p* Value[b]
Basipetal IAA	19.0 ± 1.2	13.3 ± 1.1	<0.005
Acropetal IAA	8.0 ± 0.4	8.8 ± 0.8	>0.2
Basipetal BA	7.0 ± 0.9	7.0 ± 0.8	>0.2
Normalized Basipetal IAA[c]	11.3	5.6	

[a]*The percent transport is the average and standard error of 12 separate experiments.*
[b]*The % transport in the absence and presence of 200 μM cytochalasin D is compared by Student's t* test.
[c]*Normalized basipetal IAA transport was calculated by subtracting the background diffusion (the average of the % of acropetal IAA and % basipetal BA transport) from the % basipetal IAA transport.*
Reprinted from Muday (2000), with kind permission from Kluwer Academic Publishers.

rection and acropetal IAA movement) from the amount of basipetal auxin transport. The magnitude of the effect of cytochalasin D treatment increases to twofold when the normalized values are compared (Table 1).

If an intact actin cytoskeleton is required for localization of the auxin efflux carrier complex, should fragmentation of actin filaments with cytochalasin lead to a total loss of auxin transport? This question can be considered by examination of the model in Figure 2. If the efflux carriers are totally randomized, then transport should be reduced to the level of diffusion. Basipetal IAA transport is reduced by cytochalasin D treatment but not to the level of diffusion. However, the cytochalasin D treatment was for 1 h, and this may not have been sufficient time to allow all the efflux carriers to randomize. In addition, there may have been partial recovery of polar auxin transport capacity during the 1.5-h transport period. These results are consistent with the model shown in Figure 2, although more complex possibilities cannot yet be eliminated.

To more directly examine the role of the actin cytoskeleton in localization of the auxin efflux carrier, immunocytochemical analysis of the proteins that constitute this protein complex after treatment with drugs that fragment actin will be extremely valuable. Antibodies that recognize PIN proteins, which encode a polypeptide that is predicted to be part of the efflux carrier, can be used to observe the localization. Antibodies against PIN1 and PIN2 have already been used to show the polar localization of these protein complexes (Gälweiler and others 1998; Müller and others 1998). The experimental results summarized here lead to the simple prediction that cytochalasin treatment will randomize the PIN protein localization.

Evidence for Vesicle Targeting in Establishment of the Asymmetric Localization of the Efflux Carrier

The initial establishment of the polarity of the auxin efflux carrier may require the localized targeting of vesicles. Several reports have used the drugs monesin and Brefeldin A (BFA), which are inhibitors of Golgi vesicle secretion, and examined their effects on auxin transport. Monensin reduced auxin efflux activity without reducing the amount of NPA-binding activity associated with microsomal membranes (Wilkinson and Morris 1994). This result both supported the idea that the efflux carrier has several distinct proteins, with transport and NPA binding being mediated by two different proteins and implicated vesicle targeting in controlling the localization of auxin transport proteins (Wilkinson and Morris 1994). Later experiments using Brefeldin A more clearly demonstrated that auxin efflux, and not auxin influx, depends on vesicle targeting (Delbarre and others 1998; Morris and Robinson 1998). In addition, these studies provided evidence that the efflux carrier activity is rapidly turned over, which may have important implications in the changes in auxin transport polarity in response to environmental stimuli, as discussed later (Delbarre and others 1998; Morris and Robinson 1998).

Additional experimental evidence even more strongly implicated vesicle targeting in auxin efflux carrier localization (Steinmann and others 1999). The effect of Brefeldin A on the localization of the PIN1 protein was examined in developing *Arabidopsis* lateral roots. PIN1 accumulated at the cell boundaries in untreated roots, but Brefeldin A treatment randomized this localization (Steinmann and others 1999). The localization of the PIN1 protein was also

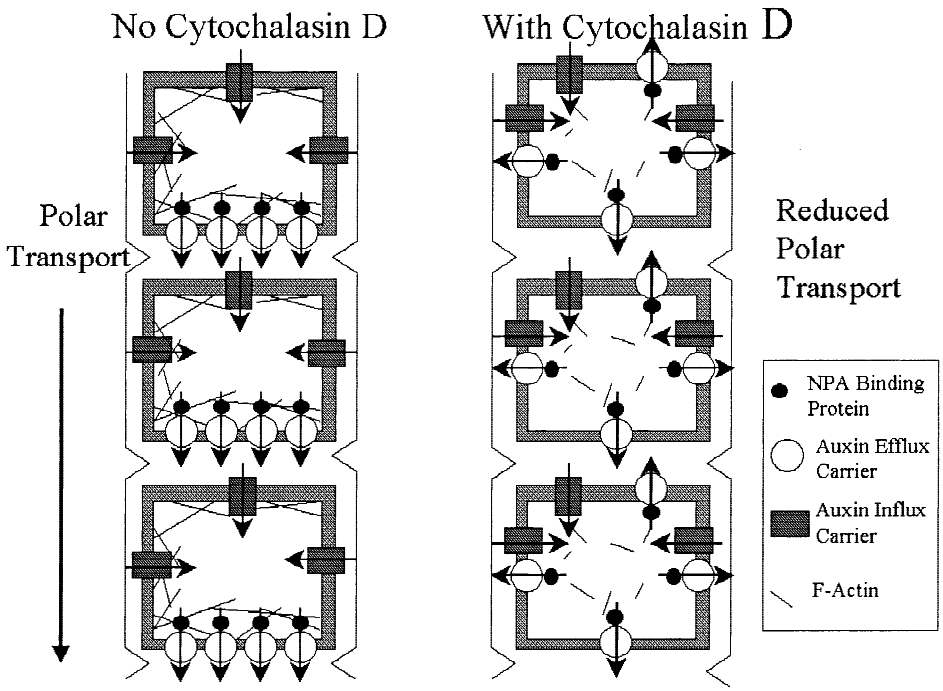

Figure 2. Model for the effect of cytochalasin D on polar auxin transport. A file of untreated cells (left) are compared with those treated with cytochalasin D (right). The fragmentation of actin filaments by cytochalasin D is shown, as well as the randomization of the auxin efflux carrier complex as a result of loss of actin structure, which may serve to localize this protein. *Reprinted from Muday (2000) with kind permission from Kluwer Academic Publishers.*

examined in embryos of the *gnom* mutant, which do not make the protein target of BFA. In the *gnom* embryos, PIN1 protein appeared disorganized with no coordinated polar localization (Steinmann and others 1999), reminiscent of the effect of BFA treatment. (Steinmann and others 1999) suggest that the BFA-sensitive GNOM protein regulates the vesicle trafficking required for the coordinated polar localization of auxin efflux carriers, which in turn determine the direction of auxin flow. Because embryos of the *gnom* mutant show defects in the apical-basal axis (Mayer and others 1993), it may be that the failure to establish polar auxin transport could lead to these axis defects. The ability of auxin transport inhibitors to cause embryo abnormalities (discussed later) that are similar to those in *gnom* mutants is consistent with this hypothesis (Steinmann and others 1999).

Together, these results suggest that integral membrane proteins of the efflux carrier move through the Golgi vesicle transport pathway and that this pathway could differentially target proteins to the basal membrane. Because most experimentation indicates that the NPA-binding protein is a peripheral membrane protein on the cytoplasmic face of the membrane (see preceding), this protein would not move through the Golgi and would presumably associate with the efflux carrier on the face of the membrane. Therefore differential targeting of vesicles containing the integral membrane proteins of the auxin efflux carrier protein complex, combined with an interaction of the entire protein complex with the actin cytoskeleton, could then explain both the establishment and maintenance of the asymmetric localization of the auxin efflux carrier protein complex.

Auxin Transport May Influence Developmental Polarity

The experimental results discussed in the preceding suggest a mechanism for the asymmetric cellular localization of the auxin efflux carrier complex through both vesicle targeting and attachment to the actin cytoskeleton. It is also possible that this auxin efflux carrier asymmetry may play an important role in the formation of developmental asymmetry. A number of recent reports using both genetic mutants and auxin transport inhibitor treatments have linked auxin transport to embryo and vascular development.

Several *Arabidopsis* mutants have been identified that have altered embryo development and appear to have a primary defect in auxin response or homeostasis. The *monopteros* mutant, which has a mutation in an auxin response factor gene (Hardtke and Berleth 1998), has both embryo and vascular developmental defects (Berleth and Jürgens 1993; Przemeck and others 1996). The vascular developmental defects of *monopteros* can also be phenocopied by growth of *Arabidopsis* plants in the presence of auxin

transport inhibitors (Mattsson and others 1999). The *bodenlos* mutant has reduced auxin sensitivity and has a similar phenotype to *monopteros* (Hamann and others 1999). The *axr6* mutants have both developmental and vascular pattern defects and are also less sensitive to applied auxins (Hobbie and others 2000). Finally, the embryo pattern mutant *fass* has defects in auxin homeostasis (Fisher and others 1996; Torres-Ruiz and Jürgens 1993). Together these results link auxin responsiveness and homeostasis to development.

Additional studies have indicated that embryo development is directly tied to the ability to properly transport auxin. Numerous studies have found that treatment of developing embryos with auxin transport inhibitors leads to abnormal pattern formation. Specifically, application of auxin efflux inhibitors led to the development of altered shapes of embryos in carrot (Schiavone 1988; Schiavone and Cooke 1987), *Ipomoea* (Chee and Cantliffe 1989), mustard (Liu and others 1993), wheat (Gälweiler and others 1998), and *Brassica* (Hadfi and others 1998). In addition, *Arabidopsis* PIN1 plants, which have a mutation in a gene predicted to encode an auxin efflux carrier (Gälweiler and others 1998), have developmental abnormalities, which are similar to those of plants that have been treated with auxin transport inhibitors (Hadfi and others 1998; Liu and others 1993). Together these studies link auxin transport to the later stages of embryo development.

The isolation of more embryo developmental mutants may provide additional insight into the link between auxin transport and embryo development. One such intriguing mutant is the maize mutant *lachrima,* which is blocked in embryogenesis at the proembryo/transition stage border. This is the same stage of embryo development that is blocked when embryos are treated with auxin transport inhibitors (Stiefel and others 1999). The recent identification of the mutated gene in *lachrima* shows that this mutation is in a gene encoding a transmembrane protein named TM20. TM20 is predicted to have 20 membrane-spanning domains and is expressed in the meristem and in developing embryos (Stiefel and others 1999). Many features of this mutant, the TM20 protein, and its expression pattern are consistent with TM20 being an auxin transport protein. Tests of this hypothesis are now possible with the isolation of the *TM20* gene (Stiefel and others 1999).

Two additional studies have examined the role of auxin transport in the early stages of embryo development. First, auxin transport in early embryogenesis was examined by inspection of the PIN1 protein localization in *Arabidopsis* embryos (Steinmann and others 1999). When the localization of the PIN1 protein is examined in wild-type embryos, polar localization becomes detectable in the midglobular stage (Steinmann and others 1999). Although this result is not consistent with auxin transport asymmetry developing at the first cell divisions in the embryo, several alternative mechanisms could mediate early asymmetries. Asymmetries in other members of the PIN gene family could occur at earlier embryo developmental stages, or early asymmetries in auxin transport may be conveyed by localized activation of a subset of auxin efflux carriers.

Additional experiments have explored the role of auxin transport in *Fucus* embryos (Basu and Muday, unpublished). In the single-celled symmetric zygotes of the brown alga, *Fucus,* embryo fertilization occurs outside the algae, so that the very earliest stages of embryo development can be readily analyzed and manipulated. The earliest evidence of *Fucus* embryo polarity is detectable within 24 h after fertilization when the site of formation of a single rhizoid becomes evident (Goodner and Quatrano 1993). Treatment of *Fucus* embryos with auxin transport inhibitors leads to formation of embryos with double rhizoids or branched rhizoids (Basu and Muday, unpublished). The auxin transport inhibitor–treated *Fucoid* embryos are reminiscent of the altered embryo structures resulting from similar treatments of somatic embryos from land plants.

In *Fucus* embryos, the site of rhizoid initiation is environmentally controlled, with light being the best-studied signal (Goodner and Quatrano 1993). As discussed later, there is a long history of experiments suggesting that the polarity of auxin movement across whole organs can change in response to light and gravity gradients. Therefore, the possibility that auxin transport also plays a role in the environmentally induced polarity of *Fucus* embryo development was examined. When *Fucus* embryos are exposed to auxin transport inhibitors, light and gravity gradients no longer control the site of rhizoid formation, but the site becomes random (Basu and Muday, unpublished).

These results suggest a role for auxin transport in the establishment of polarity during the transition from single cells to more complex embryos, yet a precise explanation for this effect is not yet possible. Changes in the organization of the actin cytoskeleton have been implicated in the early stages of *Fucus* polarity development (Alessa and Kropf 1999; Kropf and others 1989). Therefore, one possibility is that the interactions between the actin cytoskeleton and auxin transport proteins could be important in the initial stages of polarity development, because embryos go from one symmetric cell to two unequal and polar cells. It may be that either auxin move-

ments or asymmetries in auxin transport proteins are necessary for reinforcement of the signals that are needed to determine the site of rhizoid initiation (Basu and Muday, unpublished). Direct tests of these hypotheses await further experimentation.

Auxin Transport Polarity Changes in Response to Environmental Stimuli

A particularly interesting feature of auxin transport polarity is the ability of alterations in the net direction of auxin movement to occur in response to changing gradients of light and gravity. In addition to polar transport down the length of plant tissues, auxin can also move laterally across gravity or light-stimulated shoots and roots. The Cholodny-Went hypothesis, originally proposed in 1937, suggests that the lateral transport of auxin across gravity or light-stimulated plant tissues drives differential growth (Evans 1991; Muday and others 2000; Trewavas 1992). Lateral redistribution of radiolabeled IAA has been measured in both shoots (Parker and Briggs 1990) and roots (Young and others 1990), and the redistribution of IAA has been shown to precede differential growth and the gravity response (Parker and Briggs 1990). Application of auxin efflux inhibitors to growing plants completely inhibits the gravity response of roots of a number of plant species, under conditions where growth still occurs (Katekar and Geissler 1980; Muday and Haworth 1994, Rashotte and others 2000). The effect of the auxin transport inhibitor NPA on gravity response is very rapid, with application at the time of gravitropic stimulation completely inhibiting gravitropic bending (Rashotte and others 2000).

Although the validity of the Cholodny-Went hypothesis has been debated (Trewavas 1992), recent molecular and genetic evidence has provided additional support to this hypothesis (Chen and others 1999). One powerful test of this hypothesis has been through the construction of transgenic plants with an auxin responsive promoter driving the expression of β-glucuronidase. The redistribution of auxin-induced gene expression across gravity-stimulated shoots (Li and others 1991) or roots (Li and others 1999) is consistent with changes in lateral auxin transport. The ability of auxin transport inhibitors to block both differential auxin-regulated gene expression and gravitropic bending suggests that lateral auxin transport is required for differential gene expression and that localized changes in auxin sensitivity are not sufficient to control this differential growth.

Another approach that has shown the dependence of the gravity response on auxin transport has been the isolation of plants with mutations in auxin transport proteins that result in an agravitropic phenotype. The *aux1* and allelic *eir1/agr1/Atpin2/wav6* mutants have agravitropic roots (Chen and others 1999) and appear to encode auxin influx and efflux carriers, respectively (Bennett and others 1996; Chen and others 1998; Gälweiler and others 1998; Luschnig and others 1998; Marchant and others 1999; Müller and others 1998; Utsuno and others 1998). Roots of *agr1* accumulate more radiolabeled IAA than wild-type roots, which is consistent with an inhibition of auxin efflux (Chen and others 1998), whereas *aux1* roots show reduced [^{14}C] 2, 4D accumulation (Marchant and others 1999). The recent development of assays to measure polar auxin movement in the roots of *Arabidopsis* has revealed a reduction in polar auxin transport in roots of *eir1* (Rashotte and others 2000). Together, these results suggest that auxin transport plays an important role in controlling plant gravity response.

Although these results link lateral auxin transport to gravity response, some experimental results do not easily fit the simple interpretation of the Cholodny-Went hypothesis. The growth characteristics of roots in response to gravitropic stimulation have been carefully examined with computerized image analysis, and the pattern of root growth is not as simple as initially predicted (Evans 1991). The root overresponds to gravity, turning from horizontal to vertical to beyond vertical, and then growth switches from one root side to the other, to allow reorientation to the vertical (Ishikawa and others 1991). In addition, roots grown on high concentrations of auxin can still respond to gravity, even when growth is almost totally inhibited (Ishikawa and Evans 1993; Muday and Haworth 1994). These results, which appear contradictory to the Cholodny-Went hypothesis, indicate that the role of auxin in root growth and gravity response is complex, and additional experimentation will be required to completely understand how gravitropic growth is controlled (Trewavas 1992).

The mechanisms by which auxin transport can be influenced by changes in the gravity vector are still completely unknown. It is not yet clear whether the same protein complex controls auxin efflux during both polar and lateral movement of IAA. If multiple auxin efflux carrier complexes are used, then gravity-induced lateral auxin transport could require expression of a gene encoding another carrier or could be mediated by differential activation of one gene product. The presence of multiple *PIN* genes, with distinct expression patterns and subcellular localization suggests that multiple efflux carriers are likely

to be involved (Palme and Gälweiler 1999). The results from treatments with Brefeldin A suggest that the efflux carriers are rapidly turned over, which indicates that differential synthesis of alternative efflux carriers with different polar distributions could rapidly change the polarity of auxin movement (Delbarre and others 1998; Morris and Robinson 1998). In addition, differential activation of efflux carriers could also be mediated by phosphorylation, because there are several lines of experimentation that indicate auxin transport is regulated by changes in phosphorylation state of cellular proteins (Bernasconi 1996; Delbarre and others 1998; Deruere and others 1999).

An additional question that remains is whether the actin cytoskeleton plays a role in controlling lateral auxin transport. Although a role of the actin cytoskeleton in gravity response has been suggested (Sievers and others 1996), this point is not resolved. Two groups have examined the effect of cytochalasin on root gravity response (Blancaflor and Hasenstein 1997; Staves and others 1997). One group found that cytochalasin D had no effect on root gravitropism in three species (Staves and others 1997). A second report indicated that concentrations of cytochalasin B that were sufficient to fragment actin filaments in many root tissues did not affect gravitropic bending, whereas similar concentrations of cytochalasin D lead to a significantly reduced rate of gravitropic bending (Blancaflor and Hasenstein 1997). These studies have been complicated by difficulties in visualization of actin in columnella cells that are the site of root gravity perception (Blancaflor and Hasenstein 1997; Blancaflor and others 1998). Recent progress in methods for actin staining in columnella cells will be useful in demonstrating that these actin-fragmenting drugs were able to completely perturb actin in the relevant tissues (Collings and others 2000). At present, these results suggest that actin filaments are not necessary for lateral auxin transport. Identification of the proteins that mediate lateral auxin transport will allow a direct test of whether actin filaments play a role in control of this direction of auxin movement.

Conclusions

The results from these studies indicate that a regulatory subunit of the auxin efflux carrier, the NPA-binding protein, directly interacts with actin filaments. The actin association of the NPA-binding protein may localize the auxin efflux carrier complex to the plasma membrane on one end of the cell and thereby control the polarity of auxin transport. Antibodies that recognize two different isoforms of an integral membrane protein of the auxin efflux carrier, PIN proteins, show that these proteins are localized to the plasma membrane on one end of the cell (Gälweiler and others 1998; Müller and others 1998). In addition, treatment of zucchini hypocotyls and corn coleoptiles with cytochalasin B or D reduces polar auxin transport (Butler and others 1998; Cande and others 1973). It will be interesting to see whether similar treatments of plants with cytochalasin D will randomize the localization of PIN1 proteins. A randomization would be consistent with the actin cytoskeleton being required to maintain the polar distribution of the auxin efflux carrier protein complex.

Other studies in both plants and animals have demonstrated the importance of the actin cytoskeleton in maintenance of cell polarity. In yeast and the brown alga, *Fucus,* initial establishment of cell polarity requires an intact actin cytoskeleton (Goodner and Quatrano 1993; Li and others 1995) and is preceded by changes in the organization of the actin cytoskeleton (Alessa and Kropf 1999; Ayscough and Drubin 1996; Chant 1999; Kropf and others 1989). To maintain cellular polarity, a number of plasma membrane proteins that have asymmetric localization maintain their distribution by attachment to the actin cytoskeleton. In the developing zygotes of *Fucus,* the dihydropyridine receptor has been shown to develop asymmetric localization that also requires an intact actin cytoskeleton (Shaw and Quatrano 1996). Both the acetylcholine receptor of neurons, and the Na+, K+-ATPase of epithelial cells have polar distributions that are required for their function (Apel and Merlie 1995; Froehner 1993). Evidence for both of these protein complexes indicates that attachment to the actin cytoskeleton controls their localization (Froehner 1993; Nelson and Hammerton 1989). The acetylcholine receptor is a particularly interesting example, because one of the proteins in the complex is a 43-kDa peripheral membrane protein (rapsyn) that is associated with the actin cytoskeleton. In mice deficient for this protein, the acetylcholine receptor fails to localize properly (Gautam and others 1995). The NPA-binding protein may function in a similar way to localize the efflux carrier.

Although partial understanding of how and when auxin transport polarity is established and maintained is now possible, many important questions still remain. Although much evidence now suggests that auxin transport polarity is established early in development and is required for normal development, it is unclear how auxin transport may control the development of cellular or tissue level asymme-

tries. Another unanswered question is how auxin transport polarity can vary between tissues and with changing environmental gradients. Although plant shoots transport auxin in only a basipetal polarity, roots have a more complex pattern of auxin movement, with two distinct polarities occurring in two different tissues (Rashotte and others 2000; Reed and others 1998). The identification of a large *PIN* gene family with members with tissue-specific gene expression patterns and distinct localization patterns can begin to explain this complexity (Gälweiler and others 1998; Müller and others 1998; Palme and Gälweiler 1999). What is not yet clear is the mechanism by which light or gravity induces changes in the polarity of auxin transport within the same cell and/or tissue. These questions will be the direction of studies by many investigators in coming years.

ACKNOWLEDGMENTS

The critical reading of this manuscript by Jennifer Waters Shuler and Swati Basu is appreciated. This work was supported by grants from NSF (Grant No. IBN-9318250) and NASA (Grant No. NAG2-1203). Figures and some text were reprinted with kind permission from Kluwer Academic Press In Actin: A Dynamic Framework for Multiple Plant Cell Functions. (2000) C. J. Staiger, F. Baluska, D. Volkmann, and P. Barlow eds. (Dordrecht: The Netherlands) Chapter 30: Interactions between the actin cytoskeleton and an auxin transport protein by GK Muday, pages 541–556 and with kind permission from the American Society of Gravitational and Space Biology in Muday and others 2000.

REFERENCES

Alessa L, Kropf DL. 1999. F-actin marks the rhizoid pole in living Pelvetia compressa zygotes. Devel 126:201–209.

Apel ED, Merlie JP. 1995. Assembly of the postsynaptic apparatus. Curt Opin Neurobiol 5:62–67.

Ayscough KR, Drubin DG. 1996. ACTIN: general principles from studies in yeast. Annu Rev Cell Dev Biol 12:129–160.

Basu S, Brady S, Luciano R, Brian L, Quatrano R, Muday G. (In review). Environmental and developmental polariziation of *Fucus* embryos requires auxin transport. Plant Physiol

Bennett MJ, Marchant A, Green HG, May ST, Ward SP, Millner PA, Walker AR, Schulz B, Feldmann KA. 1996. Arabidopsis AUX1 gene: a permease-like regulator of root gravitropism. Science 273:948–950.

Berleth T, Jürgens G. 1993. The role of the *monopteros* gene in organising the basal body region of the *Arabidopsis* embryo. Devel 118:575–587.

Bernasconi P. 1996. Effect of synthetic and natural protein tyrosine kinase inhibitors on auxin efflux in zucchini (*Cucurbita pepo*) hypocotyls. Physiol Plant 96:205–210.

Bernasconi P, Bhavesh CP, Reagan JD, Subramanian MV. 1996. The *N*-1-naphthylphthalamic acid-binding protein is an integral membrane protein. Plant Physiol 111:427–432.

Blancaflor E, Fasano J, Gilroy S. 1998. Mapping the functional roles of cap cells in the response of Arabidopsis primary roots to gravity. Plant Physiol 116:213–222.

Blancaflor EB, Hasenstein KH. 1997. The organization of the actin cytoskeleton in vertical and graviresponding primary roots of maize. Plant Physiol 113:1447–1455.

Butler JH, Hu S, Brady SR, Dixon MW, Muday GK. 1998. *In vitro* and *in vivo* evidence for actin association of the naphthylphthalamic acid-binding protein from zucchini hypocotyls. Plant J 13:291–301.

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

Carraway C. 1992. Association of cytoskeletal proteins with membranes. In: Carraway K, Carraway C, editors. The cytoskeleton: a practical approach. Oxford: IRL Press. p 123–150.

Chant J. 1999. Cell polarity in yeast. Annu Rev Cell Dev Biol 15:365–391.

Chee RP, Cantliffe DJ. 1989. Inhibition of somatic embryogenesis in response to 2,3,5-triiodobenzoic acid and 2,4-dichlorophenoxyacetic acid in *Ipomoea batatas* (L.) Lam. cultured *in vitro*. J Plant Physiol 135:398–403.

Chen R, Hilson P, Sedbrook J, Rosen E, Caspar T, Masson PH. 1998. The *Arabidopsis thaliana AGRAVITROPIC 1* gene encodes a component of the polar-auxin-transport efflux carrier. Proc Natl Acad Sci USA 95:15112–15117.

Chen R, Rosen E, Masson PH. 1999. Gravitropism in higher plants. Plant Physiol 120:343–350.

Colledge M, Froehner SC. 1998. Signals mediating ion channel clustering at the neuromuscular junction. Curr Opin Neurobiol 8:357–363.

Collings D, Zsuppan G, Allen N, Blancaflor E. 2001. Demonstration of prominent actin filaments in the root columella. Planta 212:392–403.

Cox DN, Muday GK. 1994. NPA binding activity is peripheral to the plasma membrane and is associated with the cytoskeleton. Plant Cell 6:1941–1953.

Delbarre A, Muller P, Guern J. 1998. Short-lived and phosphorylated proteins contribute to carrier-mediated efflux, but not to influx, of auxin in suspension-cultured tobacco cells. Plant Physiol 116:833–844.

Deruere J, Jackson K, Garbers C, Soll D, Delong A. 1999. The *RCN1*-encoded A subunit of protein phosphatase 2A increases phosphatase activity *in vivo*. Plant J 20:389–399.

Dixon MW, Jacobson JA, Cady CT, Muday GK. 1996. Cytoplasmic orientation of the naphthylphthalamic acid-binding protein in zucchini plasma membrane vesicles. Plant Physiol 112:421–432.

Drubin DG, Nelson WJ. 1996. Origins of cell polarity. Cell 84:335–344.

Evans ML. 1991. Gravitropism: interaction of sensitivity modulation and effector redistribution. Plant Physiol 95:1–5.

Fisher RH, Barton MK, Cohen JD, Cooke TJ. 1996. Hormonal studies of *fass*, an Arabidopsis mutant that is altered in organ elongation. Plant Physiol 110:1109–1121.

Froehner SC. 1993. Regulation of ion channel distribution at synapses. Annu Rev Neurosci 16:347–368.

Gälweiler L, Guan C, Müller A, Wisman E, Mendgen K, Yephremov A, Palme K. 1998. Regulation of polar auxin transport by *AtPIN1* in *Arabidopsis* vascular tissue. Science 282:2226–2230.

Gautam M, Noakes PG, Mudd J, Nichol M, Chu GC, Sanes JR, Merlie JP. 1995. Failure of postsynaptic specialization to de-

velop at neuromuscular junctions of rapsyn-deficient mice. Nature 377:232–236.

Goldsmith MHM. 1977. The polar transport of auxin. Ann Rev Plant Physiol 28:439–478.

Goodner B, Quatrano RS. 1993. *Fucus* embryogenesis: a model to study the establishment of polarity. Plant Cell 5:1471–1481.

Hadfi K, Speth V, Neuhaus G. 1998. Auxin-induced developmental patterns in *Brassica juncea* embryos. Development 125:879–887.

Hamann T, Mayer U, Jürgens G. 1999. The auxin-insensitive *bodenlos* mutation affects primary root formation and apical-basal patterning in the *Arabidopsis* embryo. Development 126:1387–1395.

Hardtke C, Berleth T. 1998. The Arabidopsis gene *MONOPTEROS* encodes a transcription factor mediating embryo axis formation and vascular development. EMBO J 17:1405–1411.

Hertel R, Lomax TL, Briggs WR. 1983. Auxin transport in membrane vesicles from *Cucurbita pepo* L. Planta 157:193–201.

Hobbie L, McGovern M, Hurwitz LR, Pierro A, Liu NY, Bandyopadhyay A, Estelle M. 2000. The axr6 mutants of Arabidopsis thaliana define a gene involved in auxin response and early development. Development 127:23–32.

Hu S, Brady S, Kovar D, Staiger C, Clark G, Roux S, Muday G. 2000. Identification of plant actin binding proteins by F-actin affinity chromatography. Plant J 24:127–137.

Ishikawa H, Evans ML. 1993. The role of the distal elongation zone in the response of maize roots to auxin and gravity. Plant Physiol 102:1203–1210.

Ishikawa H, Hasenstein KH, Evans ML. 1991. Computer-based video digitizer analysis of surface extension in maize roots: kinetics of growth rate changes during gravitropism. Planta 183:381–390.

Jacobs M, Gilbert SF. 1983. Basal localization of the presumptive auxin transport carrier in pea stem cells. Science 220:1297–1300.

Katekar GF, Geissler AE. 1980. Auxin transport inhibitors: IV.evidence of a common mode of action for a proposed class of auxin transport. Plant Physiol 66:1190–1195.

Kropf DL, Berge SK, Quatrano RS. 1989. Actin localization during *Fucus* embryogenesis. Plant Cell 1:191–200.

Li R, Zheng Y, Drubin DG. 1995. Regulation of cortical actin cytoskeleton assembly during polarized cell growth in budding yeast. J Cell Biol 128:599–615.

Li Y, Hagen G, Guilfoyle TJ. 1991. An auxin-responsive promoter is differentially induced by auxin gradients during tropisms. Plant Cell 3:1167–1175.

Li Y, Wu Y, Hagen G, Guilfoyl T. 1999. Expression of the auxin-inducible GH3 promoter/GUS fusion gene as a useful molecular marker for auxin physiology. Plant Cell Physiol 40:675–682.

Liu C-M, Xu Z-H, Chua N-H. 1993. Auxin polar transport is essential for the establishment of bilateral symmetry during early plant embryogenesis. Plant Cell 5:621–630.

Lomax TL, Muday GK, Rubery P. 1995. Auxin transport. In: Davies PJ, editor. Plant hormones: physiology, biochemistry, and molecular biology. Norwell: Kluwer Academic Press. p 509–530.

Luschnig C, Gaxiola RA, Grisafi P, Fink GR. 1998. EIR1, a root-specific protein involved in auxin transport, is required for gravitropism in Arabidopsis thaliana. Genes Dev 12:2175–2187.

Marchant A, Kargul J, May ST, Muller P, Delbarre A, Perrot-Rechenmann C, Bennett MJ. 1999. *AUX1* regulates root gravitropism in Arabidopsis by facilitating auxin uptake within root apical tissues. EMBO J 18:2066–2073.

Mattsson J, Sung Z, Berleth T. 1999. Responses of plant vascular systems to auxin transport inhibition. Development 126:2979–2991.

Mayer U, Buttner G, Jürgens G. 1993. Apical-basal pattern formation in the *Arabidopsis* embryo: studies on the role of the *gnom* gene. Development 117:149–162.

Morris DA, Robinson JS. 1998. Targeting of auxin carriers to the plasma membrane: differential effects of brefeldin A on the traffic of auxin uptake and efflux carriers. Planta 205:606–612.

Morris DA, Rubery PH, Jarman J, Sabater M. 1991. Effects of inhibitors of protein synthesis on transmembrane auxin transport in *Cucurbita pepo* L. hypocotyl segments. J Exp Bot 42:773–783.

Muday G. 2000. Interactions between the actin cytoskeleton and an auxin transport protein. In: Staiger CJ, Baluska F, Volkmann D, Barlow P, editors. Actin: a dynamic framework for multiple plant cell functions. Dordrecht: The Netherlands: Kluwer Academic Press. p 541–556.

Muday G, Hu S, Brady S. 2000. The actin cytoskeleton may control the polar distribution of an auxin transport protein. Gravit Space Bio Bull 13:75–83.

Muday GK, Brunn SA, Haworth P, Subramanian M. 1993. Evidence for a single naphthylphthalamic acid binding site on the zucchini plasma membrane. Plant Physiol 103:449–456.

Muday GK, Haworth P. 1994. Tomato root growth, gravitropism, and lateral development: Correlation with auxin transport. Plant Physiol Biochem 33:193–203.

Müller A, Guan C, Gälweiler L, Tanzler P, Huijser P, Marchant A, Parry G, Bennett M, Wisman E, Palme K. 1998. *AtPIN2* defines a locus of Arabidopsis for root gravitropism control. EMBO J 17:6903–6911.

Nelson WJ, Grindstaff KK. 1997. Cell polarity: par for the polar course. Curr Biol 7:R562–564.

Nelson WJ, Hammerton RW. 1989. A membrane-cytoskeletal complex containing Na^+,K^+-ATPase, ankyrin, and fodrin in Madin-Darby canine kidney (MDCK) cells: implications for the biogenesis of epithelial cell polarity. J Cell Biol 108:893–902.

Okada K, Ueda J, Komaki MK, Bell CJ, Shimura Y. 1991. Requirement of the auxin polar transport system in early stages of *Arabidopsis* floral bud formation. Plant Cell 3:677–684.

Ortuno A, Sanchez-Bravo J, Moral JR, Acosta M, Sabater F. 1990. Changes in the concentration of indole-3-acetic acid during the growth of etiolated lupin hypocotyls. Physiol Plant 78:211–217.

Palme K, Gälweiler G. 1999. PIN-pointing the molecular basis of auxin transport. Curr Opin Plant Biol 2:375–381.

Parker KE, Briggs WR. 1990. Transport of indole-3-acetic acid during gravitropism in intact maize coleoptiles. Plant Physiol 94:1763–1769.

Przemeck GK, Mattsson J, Hardtke CS, Sung ZR, Berleth T. 1996. Studies on the role of the Arabidopsis gene MONOPTEROS in vascular development and plant cell axialization. Planta 200:229–237.

Rashotte A, Brady S, Reed R, Ante S, Muday G. 2000. Basipetal auxin transport is required for gravitropism in roots of *Arabidopsis.* Plant Physiol 122:481–490.

Reed RC, Brady SR, Muday GK. 1998. Inhibition of auxin movement from the shoot into the root inhibits lateral root development in *Arabidopsis.* Plant Physiol 118:1369–1378.

Rubery PH, Sheldrake AR. 1974. Carrier-mediated auxin transport. Planta 118:101–121.

Schiavone FM. 1988. Microamputation of somatic embryos of the domestic carrot reveals apical control of axis elongation and root regeneration. Development 103:657–664.

Schiavone FM, Cooke TJ. 1987. Unusual patterns of somatic embryogenesis in the domesticated carrot: developmental effects of exogenous auxins and auxin transport inhibitors. Cell Differ 21:53–62.

Shaw SL, Quatrano RS. 1996. Polar localization of a dihydropyridine receptor on living Fucus zygotes. J Cell Sci 109:335–342.

Sievers A, Buchen B, Hodick D. 1996. Gravity sensing in tip-growing cells. Trends, Plant Sci 1:273–279.

Staves M, Wayne R, Leopold A. 1997. Cytochalasin D does not inhibit gravitropism in roots. Am J Bot 84:1530–1535.

Steinmann T, Geldner N, Grebe M, Mangold S, Jackson C, Paris S, Gälweiler L, Palme K, Jürgens G. 1999. Coordinated polar localization of auxin efflux carrier *PIN1* by GNOM ARF GEF. Science 286:316–318.

Stiefel V, Becerra E, Roca R, Bastida M, Jahrmann T, Graziano E, Puigdomeeck P. 1999. *TM20,* a gene coding for a new class of transmembrane proteins expressed in the meristematic tissues of maize. J Biol Chem 274:27734–27739.

Sussman MR, Gardner G. 1980. Solubilization of the receptor for N-1-napthylphthalamic acid. Plant Physiol 66:1074–1078.

Torres-Ruiz R, Jürgens G. 1993. Mutations in the *FASS* gene uncouple pattern formation and embryogenesis in *Arabidopsis* development. Development 120:2967–2978.

Trewavas AJ. 1992. FORUM: What remains of the Cholodny-Went theory? Plant Cell Environ 15:759–794.

Utsuno K, Shikanai T, Yamada Y, Hashimoto T. 1998. *AGR,* an *Agravitropic* locus of *Arabidopsis thaliana,* encodes a novel membrane-protein family member. Plant Cell Physiol 39:1111–1118.

Voet JG, Howley KS, Shumsky JS. 1987. 5′-azido-*N*-1-Napthylphthalamic acid, a photolabile analog of *N*-1-Naphthylphthalamic acid. Plant Physiol 85:22–25.

Wilkinson S, Morris DA. 1994. Targeting of auxin carriers to the plasma membrane: effects of monensin on transmembrane auxin transport in *Cucurbita pepo* L. tissue. Planta 193:194–202.

Young LM, Evans ML, Hertel R. 1990. Correlations between gravitropic curvature and auxin movement across gravistimulated roots of *Zea mays.* Plant Physiol 92:792–796.

Zettl R, Feldwisch J, Boland W, Schell J, Palme K. 1992. 5′-Azido-[3,6-$^3H_2$]-1-naphthylphthalamic acid, a photoactivatable probe for naphthylphthalamic acid receptor proteins from higher plants: Identification of a 23-kDa protein from maize coleoptile plasma membranes. Proc Natl Acad Sci 89:480–484.