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Auxins and Tropisms

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ABSTRACT

Differential growth of plants in response to the changes in the light and gravity vectors requires a complex signal transduction cascade. Although many of the details of the mechanisms by which these differential growth responses are induced are as yet unknown, auxin has been implicated in both gravitropism and phototropism. Specifically, the redistribution of auxin across gravity or light-stimulated tissues has been detected and shown to be required for this process. The approaches by which auxin has been implicated in tropisms include isolation of mutants altered in auxin transport or response with altered gravitropic or phototropic response, identification of auxin gradients with radiolabeled auxin and auxin-inducible gene reporter systems, and by use of inhibitors of auxin transport that block gravitropism and phototropism. Proteins that transport auxin have been identified and the mechanisms which determine auxin transport polarity have been explored. In addition, recent evidence that reversible protein phosphorylation controls this process is summarized. Finally, the data in support of several hypotheses for mechanisms by which auxin transport could be differentially regulated during gravitropism are examined. Although many details of the mechanisms by which plants respond to gravity and light are not yet clear, numerous recent studies demonstrate the role of auxin in these processes.

Key words: Auxin; Gravitropism; Phototropism; Auxin transport; Cytoskeleton; Phosphorylation

INTRODUCTION

One of the most interesting and dramatic behaviors of plants is their ability to rapidly respond to environmental gradients with differential growth. Two of the best characterized differential growth responses are induced by gradients in light and gravity, although plants also respond to gradients in moisture (Takahashi 1997), electrical current (Wolverton and others 2000), and mechanical stimulation (Ishikawa and Evans 1992). The mechanisms by which plants perceive these light and gravity gradients, initiate a signal transduction cascade, and ultimately respond through differential growth are the focus of numerous current studies. This review will focus on the differential growth initiated in response to alterations in the gravity vector, since the role of the auxins in this process is clearly established. The connections between auxin and phototropism will also be briefly discussed.

To understand the role of auxin in gravitropic bending, it is first necessary to consider how plants perceive the gravity vector. This is a problem of physics, which is most easily solved by envisioning the gravity vector causing the settling of a dense object that then exerts pressure on a cellular structure initiating a signal transduction cascade. Although there have been extensive debates on the nature of these dense objects, starch-filled statoliths have been implicated in many plant tissues (as reviewed in Kiss 2000). In some cells the weight of the cytoplasm may also be sufficient to exert pressure on the membrane causing the initiation of gravitropic signaling (Wayne and others 1990). It is also not yet clear which cellular structure perceives the settling statolith. The plasma membrane, cytoskeleton, and

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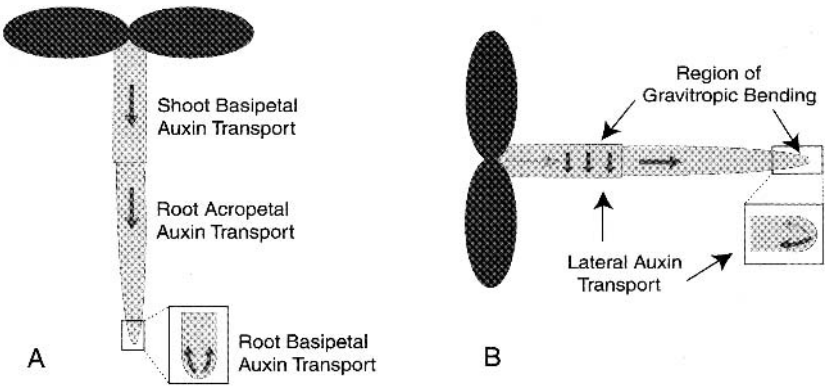

Figure 1. Auxin transport is polar in *Arabidopsis* and other plants. (**A**) In an upright hypocotyl, inflorescence, and other stem tissues, auxin moves in a single direction, from the shoot apex toward the base (basipetal). In roots, movement from the shoot into the root is from the base toward the root apex (acropetal) through cells in the central cylinder. In roots, auxin also moves from the root tip toward the base in a basipetal direction through cells of the cortex and/or epidermis. (**B**) In a plant reoriented 90° relative to gravity, although auxin transport continues in a polar fashion, lateral auxin transport also occurs. In shoot tissues this transport may occur across the hypocotyl, whereas in roots redirection of auxin transport is believed to be controlled from the root cap. The regions in which gravitropic bending will occur are also indicated.

endoplasmic reticulum have been suggested as cellular organelles that may accept the force of the settling statolith and transmit this information via a signal transduction cascade leading to altered growth (as reviewed in Sack 1991; Baluska and Hasenstein 1997).

What is quite clear about this complex process is that the ultimate response to the change in gravity vector is an alteration in the direction of plant growth. Shoots will reorient to grow against the gravity vector and roots will grow with the gravity vector. Depending on the age, size, and species of plant tissues, these growth alterations can be observed within minutes of plant reorientation and completed within hours after gravity stimulation. Although auxin was suggested to be a signal controlling this differential growth more than 100 years ago, recent evidence has provided solid support to this hypothesis.

Auxin Transport and its Role in Plant Gravity Response

Auxins, of which indole-3-acetic acid (IAA) is the predominant naturally occurring hormone, move through plants by a unique polar transport mechanism (as reviewed in Goldsmith 1977; Lomax and others 1995). This polar movement of auxin is from the shoot meristem towards the base of stems, as shown in Figure 1, and is a cell-to-cell movement. Polar auxin transport results in an auxin gradient down the length of the stem or hypocotyl, with the highest auxin concentrations found in the regions of greatest elongation (Ortuno and others 1990). In roots, auxin transport is more complex, with two distinct polarities. IAA moves acropetally (toward the root apex) through the central cylinder, and basipetally (from the apex toward the base) through the outer layers of root cells (Tsurumi and Ohwaki 1978; as reviewed in Lomax and others 1995; Jones 1998) (Fig. 1). In *Arabidopsis* roots, both of these polarities of IAA movement have been detected and linked to distinct physiological processes (Reed and others 1998; Rashotte and others 2000; Casimiro and others 2001). Basipetal auxin transport is specifically linked to root gravitropism (Rashotte and others 2000). Auxin transport is regulated and changes during development and in response to environmental stimuli (as reviewed in Lomax and others 1995).

In addition to polar transport down the length of plant tissues, auxin can also move laterally across shoots and roots stimulated by a change in the gravity vector (Fig. 1B) or in response to unilateral light. The Cholodny-Went hypothesis suggests that the lateral transport of auxin across gravity-stimulated plant tissues drives differential gravitropic growth (as reviewed in Evans 1991; 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). Additionally, gradients in endogenous-free IAA have been observed across gravity-stimulated oat and maize pulvini and maize coleoptiles (Kaufman and others 1995; Philippar and others 1999; Long and others, in press). Growth of seedlings on auxin transport inhibitors, which block auxin efflux (Rubery 1990), leads to an inhibition of the gravity response in 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, naphthylphthalamic acid (NPA), on gravity response is very rapid, with application at the time

of gravitropic stimulation completely inhibiting gravitropic bending (Rashotte and others 2000). Recently, synthetic and naturally occurring inhibitors of auxin influx have been identified and these compounds also inhibit gravitropic bending (Rahman and others 2001; Parry and others 2001).

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 (as reviewed in Chen and others 1999). One powerful test of this hypothesis has been the examination of auxin-induced gene expression across gravity-stimulated plants. First, the asymmetric accumulation of an auxin-inducible SAUR mRNA was observed in gravity-stimulated hypocotyls (McClure and Guilfoyle 1989). Transgenic plants carrying several different auxin responsive promoters driving the expression of the gene-encoding; β-glucuronidase (GUS) have now been used to show asymmetric auxin-induced gene expression across gravity-stimulated shoots (Li and others 1991; Li and others 1999) or roots (Larkin and others 1996; Luschnig and others 1998; Rashotte and others 2001). The ability of auxin transport inhibitors to block both differential auxin-regulated gene expression and gravitropic bending (Li and others 1991; Rashotte and others 2001) indicates that lateral auxin transport is required for differential gene expression. It is likely that this asymmetric gene expression also requires a change in auxin sensitivity (Salisbury and others 1988), perhaps through activation of transcription factors necessary for auxin-induced gene expression (see article by Hagen and Guilfoyle in this issue). Finally, one report of differential accumulation of a transcript of an auxin-inducible gene, *CS-IAA1,* across horizontally placed cucumber seedlings has shown that space-grown plants, which experience microgravity, fail to asymmetrically accumulate this message on the lower size of horizontal plants (Kamada and Fujii 2000). This control indicates that lateral auxin-induced gene expression is directly tied to the gravitational force.

There is also some interesting information available by careful examination of the characteristics of auxin-induced gene expression patterns in these tissues in response to gravity stimulation. When this asymmetric gene expression pattern is overlaid upon the spatial and temporal pattern of gravitropic bending, several interesting conclusions can be made. First, in shoots of tobacco, where this differential expression was first observed, the SAUR-GUS expression along the lower side of the stem extends for much of the length of the stem (Li and others 1991; Li and others 1999). The profile of regions and timing of gravity response in this tissue is not well established, but it is clear that the earliest significant changes in gene expression precede the earliest significant changes in bending (Li and others 1991).

In roots, particularly of *Arabidopsis,* there is much more information available on the spatial and temporal character of the response to gravity stimulation. Root gravitropic bending is narrowly constrained to the distal elongation zone (DEZ) (as reviewed in Evans 1991), which in a gravity-stimulated *Arabidopsis* root is localized on the lower side to a region between 100 and 300 μm from the tip (Mullen and others 1998). In the first report of differential gene expression in gravity-stimulated roots in *Arabidopsis* no temporal or spatial context to this expression pattern was reported (Luschnig and others 1998), so it is difficult to judge these differences. However, the DR5-GUS reporter detects asymmetric auxin-induced gene expression in a very local region between 100 and 250 μm from the root tip (Fig. 2) (Rashotte and others 2001). This region of asymmetric GUS expression is the basal end of the DEZ, as defined by Mullen and others (1998). The expression is also highly constrained to a narrow band of cells at the periphery of the root. This localization is consistent with previous evidence suggesting that the gravity signal in roots, presumably auxin, moves through cells of the epidermis and/or cortex (Yang and others 1990; Björkman and Cleland 1991). Additionally, in gravity-stimulated maize coleoptiles, the asymmetric localization of accumulation of a tritiated azido IAA is focused in the epidermal tissues (Jones 1990, 1992). Although GUS reporters indirectly measure changes in auxin accumulation, the ability to easily observe the expression with such high spatial resolution makes this a powerful approach to explore the role of auxin in tropisms.

What is less consistent between the Cholodny-Went hypothesis and this asymmetric DR5-GUS expression is the timing of this response relative to gravitropic bending. The DR5-GUS expression gradient is detected after roots have begun to bend (usually with a maximum at 6 h after gravity stimulation) and is detectable only in roots that have bent between 30 and 60° (Rashotte and others 2001). Similarly, the gravity-induced asymmetric induction of GH3-GUS reported in white clover also occurs concurrently with root curvature (Larkin and others 1996). In the small roots of *Arabidopsis* it is not yet possible to determine whether gradients in either free or radiolabeled auxin occur earlier than is detectable with this reporter. Studies in maize roots clearly indicate that lateral transport of radiolabeled IAA is detectable much earlier (Young and others

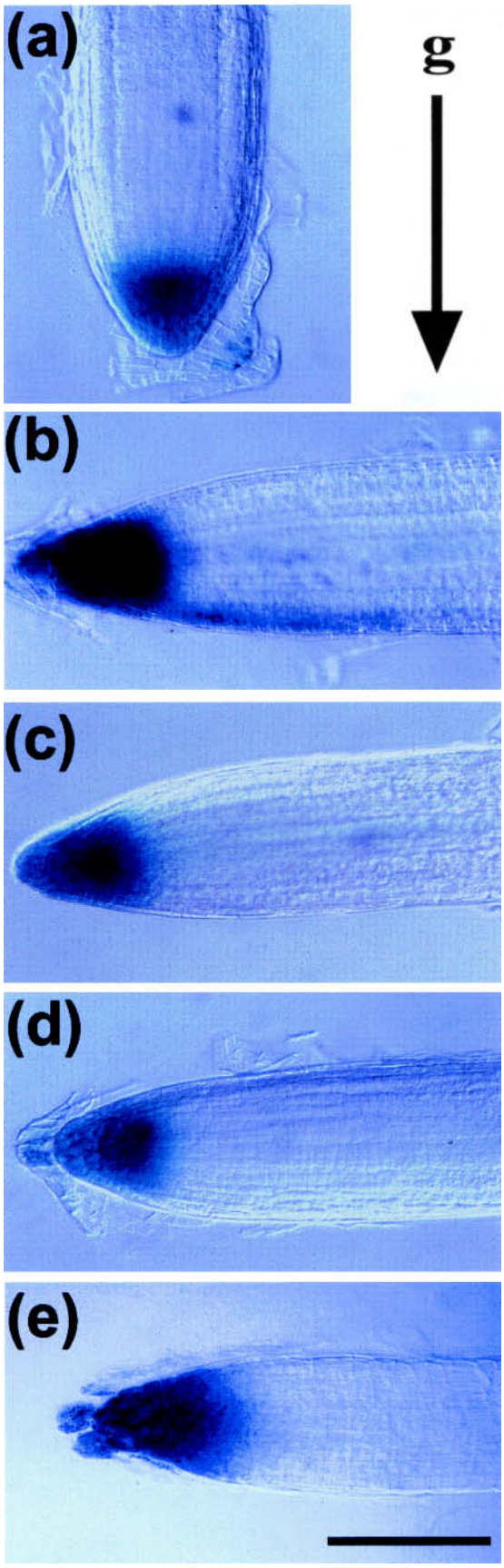

Figure 2. Expression of an auxin-responsive reporter is elevated on the lower side of gravity-stimulated wild-type roots. GUS expression was visualized in 7-day-old seedlings homozygous for the DR5-GUS reporter construct after 2-3 h of histochemical staining. Root tips are shown for (**a**) a vertically grown seedling on control media and seedlings 6 h after gravity stimulation on (**b**) control media (**c**) 1 µM NPA, (**d**) 10 µM cantharidin, and (**e**) an *rcn1* seedling carrying the DR5-GUS reporter on control media. The scale bar is equal to 100 µm. *Panels a through d reprinted from* Rashotte and others (2001), *with kind permission of the American Society of Plant Biologists.*

1990), as are gradients in free IAA in maize coleoptiles, as described above. The best explanation for this delay in auxin-induced gene expression is that the timing of the asymmetric expression reflects a lag in either activation of the machinery needed for auxin-induced gene expression or GUS protein synthesis or a higher threshold for detection of changes in auxin levels. The alternative possibility that auxin-induced GUS expression is a result of gravitropic bending is not supported by the data that indicate inhibition of auxin transport prevents plant gravity response.

Another approach that has shown the dependence of 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* mutant and the allelic *eir1/agr1/pin2/wav6* mutants have agravitropic roots and are believed to encode auxin uptake carriers (Bennett and others 1996) and auxin efflux carriers (Chen and others 1998; Luschnig and others 1998; Gälweiler and others 1998; Muller and others 1998), respectively. The inability of these plants to respond to gravity demonstrates the essential role of auxin redistribution in gravitropic bending.

Although these results link lateral auxin transport to gravity response, there are some experimental results that 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 using computerized image analysis, and the pattern of root growth is not as simple as initially predicted (as reviewed in Evans 1991). The root over responds 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). The *Arabidopsis* inflorescence has also been shown to bend past the vertical and then readjust its final position (Yamauchi and others 1997). In addition, roots grown on high concentrations of auxin can still respond to gravity,

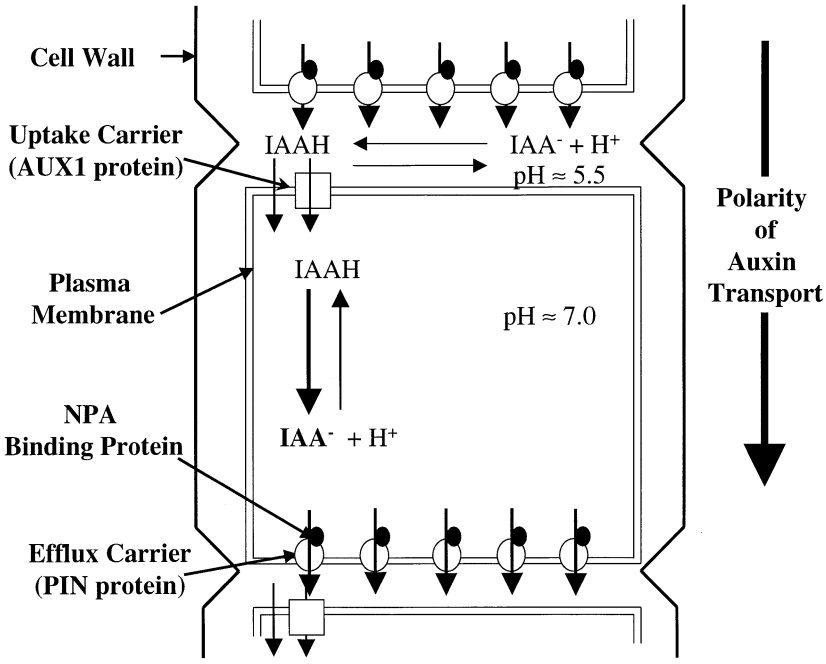

Figure 3. Schematic model of the proteins that mediate auxin transport. IAA moves into cells via an influx carrier that is believed to be encoded by the *AUX1* gene. IAA exits cells through the auxin efflux carrier complex, which contains an integral membrane catalytic component that is believed to be encoded by members of the *PIN* gene family, and a regulatory or NPA-binding subunit. *Reprinted from Muday (2000a), with kind permission from Kluwer Academic Publishers.*

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).

Auxin Transport Proteins

Identification of the proteins that transport auxin is the first step in understanding the molecular mechanisms that control both polar and gravity-induced lateral auxin transport. Recent work has provided insight into the identities of proteins that transport auxin into and out of cells (see Parry and others in this issue). These proteins are depicted in Figure 3. IAA can move into cells both passively, as it is hydrophobic when protonated, and through an influx carrier believed to be encoded by the *AUX1* gene or its homologs (Marchant and others 1999; see article by Parry and others in this issue). IAA moves out of plant cells through an efflux carrier complex that is sensitive to synthetic inhibitors of auxin transport including N-1-naphthylphthalamic acid (NPA) and triidobenzoic acid (TIBA) and is thought to be comprised of at least two polypeptides (Morris and others 1991; as reviewed in Muday 2000b). The first polypeptide of the efflux carrier is an integral membrane transporter presumably encoded by one of the members of the *PIN* gene family (Palme and Gälweiler 1999). *PIN* genes encode proteins with 10 membrane-spanning domains that are similar to other membrane transport proteins (Chen and others 1998; Gälweiler and others 1998; Luschnig and others 1998; Muller and others 1998; Utsuno and others 1998), and functional assays in yeast suggest that the *AGR1/EIR1/PIN2/WAV6* protein has auxin efflux carrier activity (Chen and others 1998; Luschnig and others 1998). Additionally, PIN proteins show an asymmetric localization in the plasma membrane, consistent with a role in controlling the polarity of auxin movement (Gälweiler and others 1998; Muller and others 1998). Several members of the *PIN* gene family in *Arabidopsis* have been identified, suggesting that there are multiple auxin efflux carriers with distinct expression patterns (as reviewed in Palme and Gälweiler 1999). Plants with mutations in either *PIN1* or *PIN2/AGRI/EIR1/WAV6* exhibit abnormal auxin transport in the inflorescence or root, respectively (Okada and others 1991; Chen and others 1998; Rashotte and others 2000), and have phenotypes consistent with these tissue-specific alterations in auxin transport (Okada and others 1991; Chen and others 1998; Luschnig and others 1998; Muller and others 1998; Utsuno and others 1998). Additionally, as discussed above, mutations in *aux1* or *agr1/eir1/pin2/wav6* lead to agravitropic root growth.

The second protein that is associated with auxin efflux carrier complexes acts as a regulatory subunit and is the binding site for auxin transport inhibitors such as NPA (Rubery 1990). Although one study has suggested an integral membrane localization for the

NPA binding protein (Bernasconi and others 1996), several other studies indicate that the NPA binding protein is a peripheral membrane protein that associates with the cytoplasmic face of the plasma membrane and is distinct from the catalytic polypeptide of the efflux carrier (Cox and Muday 1994; Dixon and others 1996; as reviewed in Muday 2000a). Under the conditions used for these studies, only a single binding site for NPA is detected (Muday and others 1993), although other investigators have identified an aminopeptidase that binds and cleaves NPA, but that activity is not detectable under these standard assay conditions (Murphy and Taiz 1999). The mechanism by which auxin transport inhibitors control auxin efflux is not known, but recent evidence suggests that the mechanisms of action of these compounds might be at the level of control of targeting of auxin transporters to the plasma membrane (Gil and others 2001; Geldner and others 2001, as summarized below.

The NPA binding protein also is believed to bind endogenous regulatory compounds such as flavonoids, which have been shown to displace NPA binding and to inhibit auxin efflux *in vitro* (Jacobs and Rubery 1988). *Arabidopsis* mutants that make no flavonoids due to a biosynthetic defect have elevated auxin transport and increased inflorescence and root branching, phenotypes consistent with increased transport (Murphy and others 2000; Brown and others 2001). The localized accumulation of flavonoids (Peer and others 2001) may function to control auxin transport *in vivo*. Changes in the synthesis or localization of endogenous regulatory molecules may alter the rate or direction of transport, working through this regulatory subunit to modulate auxin transport during gravitropic or phototropic bending (Brown and others 2001).

Regulation of Auxin Transport Protein Location and Activity During Gravity Response

To understand how auxin transport changes in response to the gravity vector, it is necessary to understand how the polarity and amount of auxin transport are regulated. Although auxin influx carriers play an important role in auxin transport and the activity of this protein is necessary for root gravitropism (see Parry and others this issue), currently there is no evidence that an auxin uptake carrier controls the direction of either polar or lateral auxin transport. In contrast, the auxin efflux carrier has been implicated in regulating the amount and direction of auxin transport (as reviewed in Lomax and others 1995). Basal localization of auxin efflux carriers has also been proposed to determine the polarity of IAA transport in plant tissues (Rubery and Sheldrake 1974; Jacobs and Gilbert 1983). Immunocytochemical approaches have demonstrated this asymmetric distribution of the PIN1 and PIN2 proteins of the efflux carrier complex in cells of the inflorescence (Gälweiler and others 1998) and root of *Arabidopsis* plants (Muller and others 1998), respectively.

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. The presence of multiple *PIN* genes, with distinct expression patterns and subcellular localizations, suggests that multiple efflux carriers are likely to be involved (as reviewed in Palme and Gälweiler 1999). Differential synthesis of alternative efflux carriers with different polar distributions or differential cycling of these carriers from the endomembrane system could rapidly change the polarity of auxin movement (Delbarre and others 1998; Morris and Robinson 1998). Additionally, differential activation of efflux carriers could also be mediated by a number of regulatory mechanisms including phosphorylation, as described below. Alternatively, changes in membrane localization could allow the same carrier to mediate both polar and lateral auxin transport of IAA.

Mechanisms That May Control Auxin Transport Polarity. The initial establishment of the polarity of auxin efflux carriers may require the localized targeting of vesicles, while maintenance of this polar localization may be mediated by attachment of auxin transport proteins to the cytoskeleton. Several reports have shown that the drugs monensin and brefeldin A (BFA), which are inhibitors of Golgi vesicle secretion, impede auxin transport (Wilkinson and Morris 1994; Morris and Robinson 1998; Delbarre and others 1998). Monensin reduces auxin efflux activity without reducing NPA binding, indicating that auxin carrier activity and NPA binding are mediated by two different proteins, and implicating vesicle targeting in controlling the localization of auxin transport proteins (Wilkinson and Morris 1994). Initial experiments using BFA suggested that auxin efflux is dependent on vesicle targeting but did not detect a role of vesicle targeting on auxin influx (Delbarre and others 1998; Morris and Robinson 1998). Additionally, these studies provided evidence that the efflux carrier activity is rapidly cycled between the Golgi and plasma membrane in the absence of protein synthesis, which may have important implications in the changes in auxin transport polarity in response to gradients of light and gravity, as discussed below (Delbarre and others 1998; Morris and Robinson 1998).

A recent study directly links vesicle targeting with localization of a putative auxin efflux carrier (Steinmann and others 1999). The effect of BFA on localization of the PIN1 protein was examined in developing *Arabidopsis* lateral roots. PIN1 accumulates at the cell boundaries in untreated roots, but BFA treatment randomizes this localization (Steinmann and others 1999). The localization of the PIN1 protein was also examined in embryos of the *gnom* mutant, which do not synthesize a protein that may be the target of BFA. In *gnom* embryos, as in BFA-treated roots, PIN1 protein exhibits no coordinated polar localization (Steinmann and others 1999). Additionally, in both the *gnom* mutant and BFA-treated seedlings, PIN1 is internalized into endomembrane compartments suggesting that this protein is either replaced from an internal compartment (Delbarre and others 1998) or cycled between the Golgi and plasma membrane (Morris 2000). 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. As *gnom* mutant embryos show apical-basal axis defects (Mayer and others 1993), it is possible that failure to establish polar auxin transport leads to these axis defects or vice-versa. The ability of auxin transport inhibitors to cause *gnom*-like embryo abnormalities (reviewed in Muday 2000b) is consistent with this hypothesis (Steinmann and others 1999).

Recent evidence has linked auxin transport to vesicle movements in a different way. Treatment with the auxin transport inhibitor TIBA prevents the effect of BFA on PIN1 localization and prevents the recovery from BFA treatment (Geldner and others 2001). In contrast, treatment with TIBA or NPA directly had little effect on PIN1 localization in wild-type roots (Geldner and others 2001), although treatment with NPA did reduce the polar localization of the PIN1 protein in roots of the *tir3* mutant, which has alterations in auxin transport (Gil and others 2001). The *tir3* mutant was isolated in a screen for reduced sensitivity to auxin transport inhibitors Ruegger and others 1997, yet it has heightened sensitivity to the action of NPA in perturbing polar localization Gil and others 2001. These results suggest that NPA and other auxin transport inhibitors may perturb the cycling of auxin efflux carriers between the plasma membrane and the endomembrane system (Estelle 2001), yet whether this is the major mechanism by which auxin transport inhibitors act is still not resolved.

Together, these results suggest that integral membrane proteins of the efflux carrier move through a vesicle transport pathway and that this pathway could differentially target proteins to the basal membrane and may recycle them to internal membrane compartments. Furthermore, auxin transport inhibitors may perturb this localization. Vesicle targeting does not appear to be important for function of the NPA binding protein (Delbarre and others 1998; Morris and Robinson 1998), consistent with the idea that this protein does not move through the Golgi but instead associates with the efflux carrier on the cytoplasmic face of the membrane (as reviewed in Muday 2000a). The biochemical behavior of the NPA binding protein has suggested that this protein interacts with the actin cytoskeleton.

Several lines of experimental evidence have suggested that the regulatory protein of the efflux carrier, an NPA binding protein, interacts with the actin cytoskeleton (as reviewed in Muday 2000a). This actin interaction may be important in localization of the efflux carrier either by facilitating movement of vesicles that deliver this protein to the plasma membrane or by maintaining the localization of the efflux carrier through attachment to the cytoskeleton. NPA binding activity is recovered in the detergent-insoluble pellet after ultracentrifugation, a behavior characteristic of proteins that interact with the cytoskeleton (Cox and Muday 1994). Furthermore, NPA binding protein is released from or stabilized in these detergent-insoluble pellets by drugs that fragment or stabilize the actin cytoskeleton, respectively (Cox and Muday 1994; Butler and others 1998). Finally, NPA binding protein associates with actin *in vitro* during rounds of actin depolymerization and repolymerization (Cox and Muday 1994).

These experiments indirectly support the hypothesis that NPA binding protein interacts with actin filaments. To assay for interaction with the actin cytoskeleton more directly, samples enriched for NPA binding activity were applied to actin affinity columns. Affinity columns containing actin monomers (G-actin) and actin filaments (F-actin), as well as columns containing BSA were constructed (Hu and others 2000). NPA binding activity is selectively retained by the F-actin column and elutes with high salt concentrations (Hu and others 2000). To date, the low abundance of this protein has prevented purification, antibody production, and sequence analysis, prohibiting some approaches to further analysis of the interaction.

The interaction between the NPA binding protein and the actin cytoskeleton may fix the auxin efflux carrier complex in one plane of the membrane or control the targeting of the efflux carrier by vesicle targeting, promoting asymmetric auxin transport. If the polarity of auxin transport is dependent on the

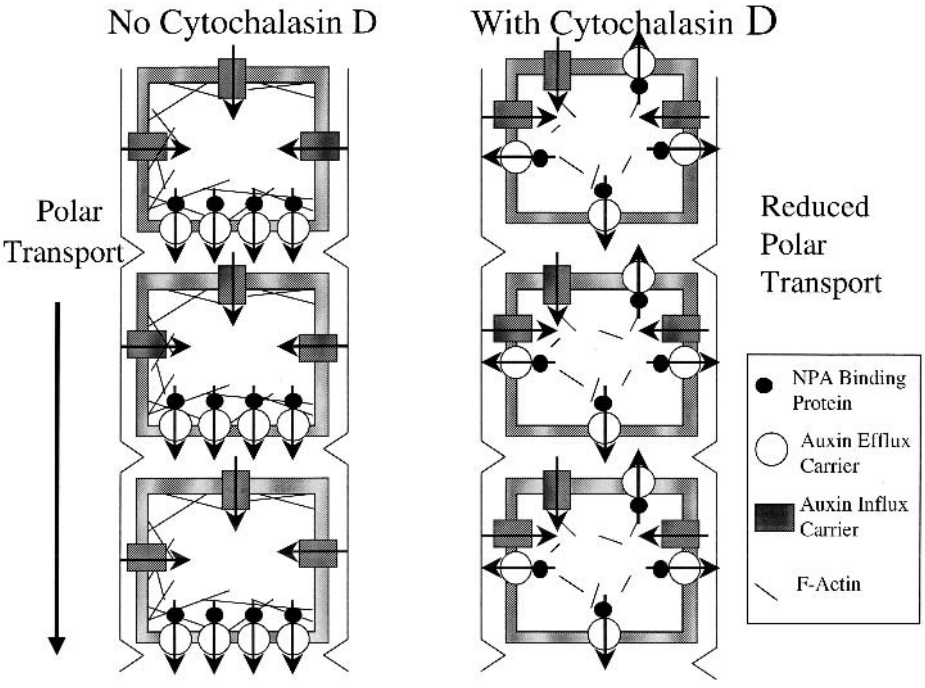

Figure 4. Model for the effect of cytochalasin D on polar auxin transport. In a file of untreated cells (left), actin filaments may serve to localize the auxin efflux carrier complex. In cytochalasin D-treated cells (right), actin filaments are fragmented, and polar auxin transport is reduced. Loss of actin structure is predicted to randomize the localization of the efflux carrier complex. *Reprinted from Muday (2000a), with kind permission from Kluwer Academic Publishers.*

actin cytoskeleton, then disruption of the actin cytoskeleton should lead to a reduction in polar auxin transport. Treatment of corn coleoptiles and zucchini hypocotyls, respectively, with cytochalasin B or D (drugs which fragment actin filaments) reduces polar auxin transport and the regulation of transport by NPA (Cande and others 1973; Butler and others 1998).

Immunocytochemical localization of PIN1 proteins after cytochalasin treatment resulted in a reduction in the polar localization of the PIN1 protein (Geldner and others 2001). These data are consistent with a requirement for cortical actin cytoskeleton in maintenance of the polar distribution of auxin transport proteins, as indicated in the model in Figure 4. Alternatively, if the function of the NPA binding protein is to mediate actin-dependent vesicle cycling between an internal membrane compartment and the plasma membrane, cytochalasin treatment might randomize PIN1 protein by inhibition of this cycling mechanism, resulting in a similar randomization of efflux carriers. The resolution of these two models awaits further experimentation.

Regulation of Auxin Transport by Reversible Protein Phosphorylation. The activity of many highly regulated proteins is controlled by phosphorylation and dephosphorylation. This mechanism is ubiquitous from bacteriophage to multicellular eukaryotes. Therefore, it is not surprising that the dynamic process of auxin transport is regulated by reversible protein phosphorylation. One *Arabidopsis* mutant that has recently provided insight into the regulation of auxin transport is *roots curl* in *NPA1* (*rcn1*). This mutant was isolated in a screen designed to identify genes encoding proteins involved in auxin transport or its regulation, using an assay for alterations in differential root elongation in the presence of NPA (Garbers and others 1996). The *RCN1* gene encodes an A regulatory subunit of protein phosphatase 2A (PP2A) and the *rcn1* mutant exhibits reduced PP2A activity in extracts, in addition to defects in root curling and other phenotypes requiring differential cell elongation (Garbers and others 1996; Deruère and others 1999). The phenotypic alterations in this mutant are consistent with reductions in PP2A activity, as treatment with the phosphatase inhibitor cantharidin produces a phenocopy of *rcn1* (Deruère and others 1999). The *RCN1* gene is expressed in the seedling root tip, the site of basipetal transport, in lateral root primordia and the pericycle and stele, the likely site of acropetal transport (Deruère and others 1999).

A recent study shows that the gravity response and basipetal auxin transport stream in seedling roots are altered by phosphatase inhibition (Rashotte and others 2001). Both the *rcn1* mutation and phosphatase inhibitor treatment of wild-type seedlings produce similar effects, arguing that PP2A activity is specifically involved in regulating transport. Basipetal transport, which has been implicated in controlling gravitropic bending (Rashotte and others 2000), is increased to over 150% of the nor-

mal wild-type level in tips of *rcn1* roots, but shows normal sensitivity to inhibition by NPA. Surprisingly, the elevated basipetal auxin transport is accompanied by a delayed gravity response (Rashotte and others 2001). The gravity response defect of *rcn1* seedlings can be partially restored by a low-dose NPA treatment. This NPA dose reduces basipetal transport in *rcn1* seedlings to nearly wild-type levels, but is sufficient to block gravitropism in wild-type seedlings. A similar treatment with an auxin transport inhibitor also restores gravitropic bending in the *hyl1* mutant (Lu and Fedoroff 2000). These data suggest that supra-optimal basipetal transport impairs gravity response, possibly by impeding formation or perception of an auxin concentration gradient across the root tip.

Consistent with this hypothesis, activity of an auxin-responsive reporter construct is altered in phosphatase-inhibited seedlings. Gravity stimulation of wild-type roots carrying the auxin-responsive DR5-GUS reporter produces an asymmetric pattern of GUS staining across the root tip that is not visible in vertical controls, as shown in Figure 2a and b (Rashotte and others 2001). Gravity response and the asymmetric DR5-GUS expression are blocked by NPA treatment (Fig. 2c). Like gravity response, formation of this asymmetric expression pattern is delayed in cantharidin-treated wild-type and *rcn1* roots, as is visible in Figure 2d and e. This asymmetric gene expression is significantly delayed in gravity stimulated *rcn1* and cantharidin-treated wild-type seedlings, and coincides with the delayed gravitropic bending of these roots. Furthermore, in plants with reduced phosphatase activity, the asymmetry of DR5-GUS expression can be partially rescued by NPA treatment (Rashotte and others 2001).

It is reasonable to assume that activity of one or more protein kinase(s) must counterbalance the effects of PP2A. Mutational analysis indicates that the genes encoding several serine/threonine kinases play key regulatory roles in processes that involve auxin transport or redistribution. NPH1 (nonphototropic hypocotyl1) and NPL1 (NPH1-like1), now renamed phototropins 1 and 2 (Briggs and others 2001), are photoreceptor kinases required for hypocotyl phototropism and other blue light dependent processes (Briggs and others 2001), a process that may require lateral auxin transport. No connections have yet been established between kinase activity and auxin transport (Liscum and Stowe-Evans 2000). The PINOID1 (PID1) kinase plays roles in development of a bilaterally symmetric embryo and in inflorescence development (Christensen and others 2000). The inflorescence phenotypes of plants carrying strong *pid1* alleles are very similar to those of plants lacking function of the auxin efflux carrier encoded by the *PIN1* gene (Bennett and others 1995). Additionally, auxin transport is reduced in several alleles of *pid1* (Bennett and others 1995), but it is not yet clear whether this is a secondary consequence of a severe loss of auxin responsiveness in affected tissues (Christensen and others 2000; DeLong and others 2001).

Inhibitor studies also have implicated protein kinases in regulation of transport. The broad-spectrum kinase inhibitors, staurosporine and K252a, rapidly reduce auxin efflux, without affecting auxin influx, in assays measuring transport of labeled auxins in cultured tobacco cells. An inhibitor of protein kinase C and a calmodulin antagonist are ineffective in these assays (Delbarre and others 1998). In contrast, the effects of tyrosine kinase inhibitors on auxin accumulation suggest that tyrosine phosphorylation may affect the NPA-binding protein rather than the auxin carrier itself, reducing the regulation of auxin efflux by NPA (Bernasconi 1996). These reports suggest that kinase activities control normal auxin efflux and its sensitivity to NPA. Inhibitors were also used to implicate protein phosphorylation in the gravitropic response of oat pulvini (Chang and Kaufman 2000), and to correlate activity of a calcium/calmodulin-dependent protein kinase with light-regulated root gravitropism in maize (Lu and Feldman 1997). However, the mechanistic roles of the inhibitor targets in these latter studies are entirely unknown.

Further experiments will be required to determine the mechanism by which PP2A affects auxin transport in roots, and to identify the targets of kinase and phosphatase regulation in auxin transport. Some possible targets for regulation by phosphorylation are the influx and efflux carrier proteins themselves. However, double mutant analyses indicate that elevated basipetal transport in *rcn1* or cantharidin-treated wild-type seedlings does not require the products of the *AGR1/EIR1/PIN2* or *AUX1* genes, indicating that PP2A is unlikely to act directly on those proteins (Rashotte and others 2001). Loss of RCN1 function might allow activation of an efflux carrier that is normally inactive in basipetal transport. A second potential point of regulation may be the NPA binding protein. Alternatively, phosphorylation might affect transport by controlling biosynthesis of endogenous regulatory compounds such as flavonoids (Brown and others 2001). One report indicates that protein phosphatase inhibitors prevent the light induced expression of a gene encoding a flavonoid biosynthetic enzyme (Christie and Jenkins 1996). Finally, since phosphorylation is known to influence cytoskeletal organization (Baskin and Wil-

son 1997), it is also possible that phosphorylation may provide an additional and overlapping regulatory mechanism at the level of localization of carrier complexes. In animal cells there are specific membrane-associated actin-binding proteins that are the target of phosphorylation and control the organization of the actin cytoskeleton (Bretscher 1999). An intriguing, but unexplored, possibility is that similar proteins function in plant cells to organize actin networks, including those that localize proteins such as the auxin efflux carrier to specific membrane domains, as discussed in the following section.

Regulation of Auxin Transport by Other Signaling Pathways. Biochemical and cell biological approaches designed to test for gravity induced changes in potential signaling molecules have provided candidates that could control the activity of auxin transport proteins. It has long been suspected that calcium is a signaling molecule that changes in concentration in response to changes in the vector of gravity (as reviewed in Sinclair and Trewavas 1997). Recent attempts to demonstrate changes in cytoplasmic calcium concentration using calcium ratio imaging in *Arabidopsis* roots have not detected calcium concentration changes in response to gravitropic stimulation (Legue and others 1997). Several experiments have suggested that gravity stimulation may be amplified by cascades involving Ca^{2+}/calmodulin (Sinclair and others 1996; Lu and Feldman 1997) and a number of older studies suggested a relationship between auxin transport and calcium concentration (DelaFuente 1984; Allan and Rubery 1991). Therefore, the role of calcium in gravity response awaits resolution.

The role of several other signaling molecules in gravitropism has been more clearly demonstrated. Changes in pH have been observed after gravitropic stimulation in both *Arabidopsis* roots (Scott and Allen 1999; Fasano and others 2001) and the maize pulvinus (Johannes and others 2001). Additionally, changes in inositol lipid signaling have been observed in gravity-stimulated maize and oat pulvini (Perera and others 1999; Perera and others 2001). Gravity-stimulated pulvini undergo rapid initial changes in IP_3 levels on both sides, followed by a greater and more persistent elevation on the lower side (Perera and others 1999). This later IP_3 elevation on the lower side is necessary for gravitropic bending of the pulvinus, as treatment with phospholipase C inhibitors prevents formation of the IP_3 gradient and reduced gravitropic bending (Perera and others 1999). In maize, free IAA has been measured and shown to develop an asymmetry across the gravity stimulated pulvinus that follows the changes in IP_3 levels (Long and others, in press). The identification of a number of signaling molecules that change during gravity stimulation in the maize pulvinus suggests that this will be an excellent model system to examine biochemical changes in response to gravity stimulation. Future experiments will test whether either the pH or IP_3 changes detected in response to gravitropic stimulation are used to change the activity or localization of auxin transport proteins and thereby lead to differential gravitropic growth.

The Role of the Cytoskeleton in Gravity Perception and Response

The idea that the cytoskeleton could function in translating the physical force of gravity into altered growth is interesting, but as yet, unproven. A role of the cytoskeleton in gravity perception and or response has been explored by several authors (as reviewed by Sievers and others 1996; Baluska and Hasenstein 1997), but this point is not resolved. A number of studies have directly explored the possibility that the cytoskeleton is involved in the auxin-mediated differential growth in gravity-stimulated tissues (as reviewed in Baluska and Hasenstein 1997). Several groups have observed changes in the organization of the microtubule (MT) cytoskeleton in gravity-stimulated maize roots or coleoptiles (Nick and others 1990; Blancaflor and Hasenstein 1993; Himmelspach and others 1999) and these MT changes can be mimicked by auxin treatment (Himmelspach and others 1999; Blancaflor and Hasenstein 1995; Takesue and Shibaoka 1998). In roots, treatment with microtubule depolymerizing drugs does perturb the growth and gravity response, but the timing of this effect may be delayed until after gravity response is initiated (Baluska and others 1996; Blancaflor and Hasenstein 1995). Also, the effects of microtubule stabilizing and depolymerizing drugs on auxin transport in maize roots were examined and no changes were observed in response to treatments that altered MT organization (Hasenstein and others 1999). In contrast, in coleoptiles, the microtubule reorientation induced by gravitropic stimulation precedes changes in growth and gravitropic bending (Himmelspach and Nick 2001), and treatment with microtubule depolymerizing drugs led to an inhibition of lateral auxin transport (Godbole and others 1999). Perhaps these different findings result from different mechanisms controlling gravitropic bending in the root and coleoptile.

The function of the actin cytoskeleton in gravity response is less well studied. The evidence that one auxin transport protein interacts with actin, summarized above, suggests a model in which auxin trans-

port polarity could be changed in response to gravity stimulation by reorganization of actin, but this hypothesis has not been experimentally tested. Examination of the organization of actin filaments in gravity-stimulated maize roots lead to no significant changes in organization (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 examining actin organization in greater detail (Collings and others 2001).

Several inhibitor studies have not detected a role for the actin cytoskeleton in gravity response. Several groups have examined the effect of cytochalasin, a drug that fragments the actin cytoskeleton, 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 partial reductions in gravitropic bending (Blancaflor and Hasenstein 1997). In rice coleoptiles, cytochalasin D was also unable to perturb gravitropic bending or gravity-induced lateral auxin transport (Godbole and others 2000).

The recent isolation of several mutants has suggested a connection between the cytoskeleton and gravity response. The *arg1* mutant has a defect in a gene that encodes a protein with a DnaJ domain, which is used by a number of proteins that interact with the cytoskeleton (Sedbrook and others 1999). Coleoptiles from the rice mutant *Yin-Yang* exhibit a more rapid initiation of gravitropic bending, a slower rate of gravitropic bending, and then continue to bend past the vertical (Wang and Nick 1998). These gravitropic alterations can be mimicked by treatment with cytochalasin D. Finally, in the *Yin-Yang* mutant, actin microfilaments become depolymerized in response to auxin treatment (Wang and Nick 1998).

Currently, these results do not clearly answer the question of whether the cytoskeleton is necessary for gravity-induced lateral auxin transport. Identification of the proteins that mediate lateral auxin transport will allow a direct test of whether either actin filaments or microtubules control the initiation of lateral auxin transport in response to gravity stimulation.

Isolation of Mutants with Altered Gravity Response

The advent of *Arabidopsis* molecular genetics has been particularly fruitful in understanding many processes, including gravitropism. A number of *Arabidopsis* mutants have been isolated with altered gravity response and many have pointed to an important role of auxin in this process. First, a number of mutants have been implicated in gravity perception. These mutants have altered starch content (Kiss 2000), defects in amyloplast synthesis (Fujihira and others 2000), or altered development of the tissues that contain sedimenting amyloplasts (Fukaki and others 1998; Tasaka and others 1999). The reduction or loss of gravity response in these mutants is strong support for the hypothesis that amyloplasts act as statoliths that settle in response to a new gravity vector and thereby initiate a signal transduction cascade.

The most abundant set of mutants with altered gravity response have mapped to either the auxin transport or auxin signaling pathways. As discussed above, mutants in either *aux1,* a gene encoding a putative auxin influx carrier, or *agr1/eir1/pin2/wav6,* a gene encoding a putative auxin efflux carrier, lead to agravitropic roots indicating that transport of auxin is essential for proper root gravitropism (as reviewed in Chen and others 1999). Several interesting tomato mutants with gravitropic phenotypes have been isolated that include *dgt,* with altered auxin physiology (Rice and Lomax 2000; Nebenfuhr and others 2000), and *lazy-2,* with altered light-dependent gravity response (Gaiser and Lomax 1993). Additionally, the *Lazy* mutation in rice has been shown to have reduced lateral transport of auxin after gravity stimulation, although polar auxin transport in *Lazy* coleoptiles is not affected (Godbole and others 1999). The molecular character of these mutations is not yet known.

Second, a number of mutants that were isolated for impaired growth inhibition by auxin have been found to have altered root gravitropism (as reviewed in Estelle and Klee 1994). These mutants include *aux1, axr1* to *axr4, axr6,* and *dwf.* The *axr* mutants all have defects in root gravitropism, although the defects are relatively minor in *axr1, axr4,* and *axr6,* but quite severe in *axr2, axr3, aux1,* and *dwf* (Estelle and Klee 1994; Hobbie and others 2000). The molecular defects in these mutants are summarized and their role in auxin signaling is reviewed in this issue by Ward and Estelle. Additionally, one mutant that is insensitive to another naturally occurring auxin, indole-butyric acid (IBA), has been isolated and has

reduced root gravitropic bending (Poupart and Waddell 2000).

Mutants have also been isolated with impaired growth responses to auxin transport inhibitors and impaired root gravitropic bending including *tir1* to *tir5* (Ruegger and others 1997; Ruegger and others 1998), *pis1* (Fujita and Syono 1997) and *rcn1* (Garbers and others 1996; Rashotte and others 2001). The molecular defect in the *tir1* mutant is also discussed by Ward and Estelle in this issue. The *tir3* mutant has recently been found to be allelic to *doc1* and the altered gene in these two mutants has been isolated and renamed *BIG,* because of the large size of the encoded gene product (Gil and others 2001). The *tir3/doc*1 mutant also has altered regulation of gene expression in the dark. The *tir5* mutation has been found to be allelic to *nph4,* which leads to impaired phototropic and gravitropic hypocotyl growth, which is summarized below (Liscum and Stowe-Evans 2000). Although roots of the *pis1* mutant are normally gravitropic, the mutant is hypersensitive to NPA and becomes agravitropic at lower concentrations of NPA than wild-type. The altered sensitivity applies to the inhibitors NPA and TIBA, but not to HFCA (Fujita and Syono 1997). The *ifl1* mutant was isolated for alterations in differentiation of interfascicular fibers and seconday xylem and has been found to have an alteration in auxin transport, although the gravitropic behavior of this mutant has not been reported (Zhong and Ye 2001).

Screens directly aimed at isolation of mutants with altered gravitropism have yielded a number of interesting mutations, including some of the auxin mutants described above. The *arg1* mutant is not impaired in sensitivity to auxins or auxin transport inhibitors but it has been suggested to be a signal transduction mutant (Sedbrook and others 1999). The molecular cloning of the *ARG1* gene suggests that this protein might function in protein-protein interactions, perhaps with the cytoskeleton (Sedbrook and others 1999). The *gps1* to *gps3* mutants were identified in a mutant screen in which perception and gravity response were uncoupled in the inflorescence by a cold treatment, with the goal of identifying gravitropic signaling components (Wyatt and others, personal communication). All three *gps* mutants have similar phenotypes in the hypocotyl and inflorescence, but normal root gravitropism. The *sgr4,sgr5,* and *sgr6* mutants were identified in a screen for altered gravitropic bending in the inflorescence stem (Yamauchi and others 1997). These plants have a normal phototropic response in all tissues and normal root gravitropism. The *sgr4-1* mutant has a gravitropism defect in hypocotyls, as well as inflorescence, in contrast to *sgr5-1* and *sgr6-1,* which have normal hypocotyl gravitropism. Because all three of these mutants have normal starch accumulation, and normal phototropic response, it has been suggested that these defects are due to a step in between gravity perception and differential growth (Yamauchi and others 1997). In contrast, in the *rhg* mutant, roots and hypocotyls are both agravitropic whereas inflorescences are not (Fukaki and others 1997). This mutant also has normal starch and normal auxin sensitivity, suggesting it also has a defect in signal transduction (Fukaki and others 1997). Recently, the *clg1* mutant was isolated based on its altered root growth, including agravitropic roots (Ferrari and others 2000). The *clg1* roots are subtly changed in sensitivity to auxins and auxin transport inhibitors, but have a more pronounced insensitivity to ethylene.

The *hyl1* mutant has a number of growth and developmental phenotypes including reduced gravitropic response (Lu and Fedoroff 2000). The *hyl1* plants are less sensitive to growth inhibition by both auxin and cytokinin, but are hypersensitive to ABA. Just as in the case with the *rcn1* mutant (Rashotte and others 2001), treatment of the *hyl1* mutant with an auxin transport inhibitor partially restores root gravitropism, although direct measurements of auxin transport in *hyl1* have not yet been reported (Lu and Fedoroff 2000). The *HYL1* gene encodes a protein with double-stranded RNA binding motifs and has been suggested to be a regulatory protein with transcriptional or post-transcriptional control (Lu and Federoff 2000).

Auxins and Phototropism

Just as plants bend in response to changes in the gravity vector, as summarized above, both shoots and roots will undergo differential growth in response to light gradients (Liscum and Stowe-Evans 2000; Vitha and others 2000; Ruppel and others 2001). Although the general idea that lateral redistribution of auxin is a necessary component of the phototropic response is not new, the relationship between these two processes appears complex. Although a number of investigators have detected lateral movement of radiolabeled auxin or of endogenous IAA or have observed gradients in auxin induced gene expression in response to unidirectional light in coleoptiles of maize and rice (Baskin and others 1986; Iino 1991, Li and others 1991), the inability of some investigators to detect auxin gradients in response to unilateral light (Sakoda and Hasegawa 1989) has made for interesting debates on this subject (as reviewed in Trewavas 1992). Some investigators have argued that phototropism causes

a redistribution of a growth inhibitor to the lighted side, rather than redistribution of a growth-promoting substance, such as auxin, to the dark side (Bruinsma and Hasegawa 1990). The ability of NPA to block hypocotyl phototropism in *Arabidopsis* (Harper and others 2000), suggests that auxin redistribution is part of phototropism. Furthermore, light and auxin may interact in control of other developmental processes in plants and these connections are reviewed in this issue by Tian and Reed.

One important difference between light and gravity-induced auxin gradients has been reported. During gravitropic bending in maize coleoptiles, lateral translocation of IAA has been reported to occur along the length of the coleoptile, whereas during phototropism, lateral auxin movement occurred only at the coleoptile tip (Iino 1995). In one recent study, free IAA was measured by GC-MS across gravity-stimulated maize coleoptiles for up to 1 h after gravity stimulation (Philippar and others 1999). In that report, gradients of free IAA are observed in the first 2 mm of the coleoptile, but the distance further down the length of the coleoptile or at later time points was not examined (Philippar and others 1999), preventing complete comparison of these two works. This potential difference between light- and gravity-induced auxin gradients may reflect the sites of perception of these two stimuli or fundamental differences in how auxin transport is regulated.

A number of recent advances in isolation of mutants have provided additional understanding of the process of phototropism and the complexities of the interactions among light, gravity, and auxin in phototropic growth. The light receptors that control phototropic bending have been isolated and some of the signal transduction events that control phototropism have now been identified (Liscum and Stowe-Evans 2000; Briggs and others 2001). The identification of these proteins and isolation of mutants without phototropic bending have greatly strengthened the case for the participation of auxin in this process. The phototropin photoreceptor is encoded by the *NPH1* (non-phototropic hypocotyl) gene and is a blue light-dependent kinase (as reviewed in Liscum and Stowe-Evans 2000). Null mutants in *NPH1* do not have a phototropic response to low fluence blue light, but still bend in response to high fluence light (Liscum and Stowe-Evans 2000). Therefore, another photoreceptor appears to participate in this process in this high fluence response and has been recently demonstrated to be NPL1 (Sakai and others 2001). Additionally, both cryptochrome and photochrome receptors have been implicated in this process (Ahmad and others 1998; Stowe-Evans and others 2001). The targets of the phototropin kinase are not yet known, although the first step in activation of this receptor has been clearly shown to be autophosphorylation (Liscum and Stowe-Evans 2000). Additional proteins that function in phototropism have been identified in the same mutant screen that yielded the *nph1* mutant. The *nph3* and *nph4/msg1/tir5* mutants appear to be altered in other aspects of signal transduction. The NPH3 protein has been suggested to function in maintenance of the phototropin receptor protein complex as a scaffolding protein (Stowe-Evans and others 2001).

The most interesting of these phototropic mutants, in the context of auxin, is the *nph4/msg1/tir5* mutant. This mutant was isolated in several screens including loss of phototropism in hypocotyls (Stowe-Evans and others 1998), loss of auxin-induced growth curvature (Watahiki and Yamamoto 1997), and insensitivity to auxin transport inhibitors (Ruegger and others 1997). This mutant has reductions in both phototropic and gravitropic bending, apical hook maintenance, auxin sensitivity to growth regulation, and auxin-regulated gene expression (Stowe-Evans and others 1998). The NPH4 gene has now been cloned and shown to encode ARF7 (Auxin Response Factor 7), a gene encoding a transcription factor that controls expression of a number of auxin-regulated genes (Harper and others 2000). The isolation of this mutant has clearly shown that auxin response is needed for phototropism. The mechanisms by which light signals are transmitted to auxin signals are not yet clear. Several reports indicate that the protein products of Aux/IAA genes, whose transcription are rapidly induced by auxin, may physically interact with phytochrome (Soh 1999) and may be substrates for the kinase activity of phytochrome (Colon-Carmona and others 2000).

Mechanism by Which Auxin Induces Differential Cell Elongation

The results summarized above indicate that auxin is redistributed across tissues that have changes in the gravity vector or unilateral light. The final question that remains to be answered is how does the lateral distribution of auxin stimulate growth. Some of the earliest events in response to elevated auxin are at the transcriptional level, in which auxin-induced genes are synthesized by auxin-specific transcription factors (as reviewed in this issue by Guilfoyle and Hagen). Additionally, there are a number of ionic and metabolic changes that stimulate growth and loosen the cell wall (as reviewed by Cosgrove 1997). Several recent studies beyond those summarized in

this review are particularly relevant. The acid growth theory has suggested that the wall is loosened by protons (Rayle and Cleland 1992). Recent studies have identified changes in pH in both the cytoplasm and apoplast in response to gravity stimulation (Scott and Allen 1999; Fasano and others 2001). Additionally, a potassium channel has recently been shown to be synthesized during gravity response and induced by auxin (Philippar and others 1999). A number of enzymes that act in loosening the cell wall have now been identified. The activities of expansins are pH dependent and these enzymes are particularly logical candidates as the protein that transduces the pH asymmetry into a growth asymmetry during gravitropism (as reviewed by Cosgrove 1997). Another requirement for differential growth is energy to power the process. A recent report indicates differential expression of a gene encoding a sucrose cleaving invertase across a gravity-stimulated pulivinus (Long and others, in press). The abundance of the invertase RNA is regulated by auxin and gradients in invertase RNA parallel the gradients in auxin across the gravity-stimulated pulvinus (Long and others, in press).

Conclusions

The ability of plants to change their growth orientation in response to gradients in light and gravity maximizes their ability to obtain energy from light and moisture and nutrients from soil. The evidence for a central role for auxin in control of the differential growth patterns of plants has been summarized here. Of particular relevance is the idea and emerging data that auxin transport is changed in orientation to allow these growth processes to occur. Current studies are focused on the understanding of the mechanisms by which auxin transport can be regulated to allow changes in direction of auxin transport. Finally, the mechanisms by which auxin stimulates growth are the focus of additional studies reviewed throughout this issue. Together these studies will strive to provide a complete picture of the molecular mechanisms by which auxin regulates differential growth of plants in response to a changing environment.

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REFERENCES

Ahmad M, Jarillo JA, Smirnova O, Cashmore AR. 1998. Cryptochrome blue-light photoreceptors of Arabidopsis implicated in phototropism. Nature 392:720–723. DOI: 10.1038/33701

Allan AC, Rubery PH. 1991. Calcium deficiency and auxin transport in *Cucurbita pepo* 1. Seedlings. Planta 183:604–612.

Baluska F, Hasenstein K. 1997. Root cytoskeleton; its role in perception of and response to gravity. Planta 203:S69–S78.

Baluska F, Hauskrecht M, Barlow P, Sievers A. 1996. Gravitropism of the primary root of maize: a complex pattern of differential cellular growth in the cortex independent of the microtubular cytoskeleton. Planta 198:310–318.

Baskin T, Briggs W, Iino M. 1986. Can lateral redistribution of auxin account for phototropism in maize coleoptiles? Plant Physiol 81:306–309.

Baskin TI, Wilson JE. 1997. Inhibitors of protein kinases and phosphatases alter root morphology and disorganize cortical microtubules. Plant Physiol 113:493–502.

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.

Bennett SRM, Alvarez J, Bossinger G, Smyth DR. 1995. Morphogenesis in *pinoid* mutants of *Arabidopsis thaliana*. Plant J 8:505–520.

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, Patel BC, Reagan JD, Subramanian MV. 1996. The N-1-naphthylphthalamic acid-binding protein is an integral membrane protein. Plant Physiol 111:427–432.

Björkman T, Cleland RE. 1991. Root growth regulation and gravitropism in maize roots does not require the epidermis. Planta 185:34–37.

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. 1993. Organization of cortical microtubules in graviresponding maize roots. Planta 191:231–237.

Blancaflor EB, Hasenstein KH. 1995. Time course and auxin sensitivity of cortical microtubule reorientation in maize roots. Protoplasma 185:72–82.

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

Bretscher A. 1999. Regulation of cortical structure by the ezrin-radixin-moesin protein family. Curr Opin Cell Biol 11:109–116. DOI: 10.1016/S0955-0674(99)80013-1

Briggs W, Beck C, Cashmore A, Christie J, Hughes J, Jarillo J, , Kagawa Tague B, Kanegae H, Liscum E, Nagatani A, Okada K, Salomon M, Rudiger W, Sakai T, Takano M, Wada M, Watson J. 2001. The phototropic family of photoreceptors. Plant Cell 13:993–998. DOI: 10.1105/tpc.13.5.993

Brown D, Rashotte A, Murphy A, Tague B, Peer W, Taiz L, Muday G. 2001. Flavonoids act as negative regulators of auxin transport *in vivo* in *Arabidopsis thaliana*. Plant Physiol 126:524–535. DOI: 10.1104/pp.126.2.524

Bruinsma J, Hasegawa K. 1990. A new theory of phototropism - its regulation by a light-induced gradient of auxin-inhibiting substances. Physiol Plant 79:700–704.

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.

Casimiro I, Marchant A, Bhalerao RP, Beeckman T, Dhooge S, Swarup R, Graham N, Inze D, Sandberg G, Casero PJ, Bennett M. 2001. Auxin transport promotes Arabidopsis lateral root initiation. Plant Cell 13:843–852. DOI: 10.1105/tpc.13.4.843

Chang S, Kaufman P. 2000. Effects of staurosporine, okadaic acid and sodium fluoride on protein phosphorylation in gravireSponding oat shoot pulvini. Plant Physiol Biochem 38:315–323. DOI: 10.1016/S0981-9428(00)00745-2

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. DOI: 10.1073/pnas.95.25.15112

Chen R, Rosen E, Masson PH. 1999. Gravitropism in higher plants. Plant Physiol 120:243–250.

Christensen SK, Dagenais N, Chory J, Weigel D. 2000. Regulation of auxin response by the protein kinase *pinoid.* Cell 100:469–478.

Christie JM, Jenkins GI. 1996. Distinct UV-b and UV-a/blue light signal transduction pathways induce chalcone synthase gene expression in arabidopsis cells. Plant Cell 8:1555–1567.

Collings DA, Zsuppan G, Allen NS, Blancaflor EB. 2001. Demonstration of prominent actin filaments in the root columella. Planta 212:392–403.

Colon-Carmona A, Chen DL, Yeh KC, Abel S. 2000. AUX/IAA proteins are phosphorylated by phytochrome in vitro. Plant Physiol 124:1728–1738.

Cosgrove D. 1997. Cellular mechanisms underlying growth asymmetry during stem gravitropism. Planta 203:S130–S135.

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.

DelaFuente RK. 1984. Role of calcium in the polar secretion of indoleacetic acid. Plant Physiology 76:342–346.

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.

DeLong A, Mockaitis K, Christensen S. 2001. Protein phosphorylation in the delivery of and response to auxin signals. Plant Mol Bio : (in press)

Deruère J, Jackson K, Garbers C, Soll D, Delong A. 1995. The *RCN1*-encoded subunit of protein phosphatase 2A increases phosphatase activity *in vivo.* Plant J 20:389–399. DOI: 10.1046/j.1365-313X.1999.00607.x

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.

Estelle M, Klee H. 1994. Auxin and cytokinin in *arabidopsis.* In: Meyerowitz E, editor. Arabidopsis. Cold Spring Harbor: Cold Spring Harbor Laboratory Press. p 555–578.

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

Fasano JM, Swanson SJ, Blancaflor EB, Dowd PE, Kao T, Gilroy S. 2001. Changes in root cap pH are required for the gravity response of the arabidopsis root. Plant Cell 13:907–922. DOI: 10.1105/tpc.13.4.907

Ferrari S, Piconese S, Tronelli G, Migliaccio F. 2000. A new *Arabidopsis thaliana* root gravitropism and chirality mutant. Plant Sci 158:77–85. DOI: 10.1016/S0168-9452(00)00309-5

Fujihira K, Kurata T, Watahiki MK, Karahara I, Yamamoto KT. 2000. An agravitropic mutant of Arabidopsis, *endodermal-amyloplast less 1,* that lacks amyloplasts in hypocotyl endodermal cell layer. Plant Cell Physiol 41:1193–1199.

Fujita H, Syono K. 1997. *pis1,* a negative regulator of the action of auxin transport inhibitors in *Arabidopsis thaliana.* Plant J 12:583–595.

Fukaki H, Fujisawa H, Tasaka M. 1997. The *rhg* gene is involved in root and hypocotyl gravitropism in Arabidopsis thaliana. Plant Cell Physiol 38:804–810.

Fukaki H, Wysocka-Diller J, Kato T, Fujisawa H, Benfey PN, Tasaka M. 1998. Genetic evidence that the endodermis is essential for shoot gravitropism in *Arabidopsis thaliana.* Plant J 14:425–430.

Gaiser JC, Lomax TL. 1993. The altered gravitropic response of the *lazy-2* mutant of tomato is phytochrome regulated. Plant Physiol 102:339–344.

Gälweiler L, Guan C, Muller 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.

Garbers C, DeLong A, Deruère J, Bernasconi P, Soll D. 1996. A mutation in protein phosphatase 2A regulatory subunit a affects auxin transport in Arabidopsis. EMBO J 15:2115–2124.

Gil P, Dewey E, Friml J, Zhao Y, Snowden K, Putterill J, Plame K, Estelle M, Chory J. 2001. BIG, a calossin-like protein required for polar auxin transport in *Arabidopsis.* Genes Dev : (in press)

Godbole R, Michalke W, Nick P, Hertel R. 2000. Cytoskeletal drugs and gravity-induced lateral auxin transport in rice coleoptiles. Plant Biol 2:176–181.

Godbole R, Takahashi H, Hertel R. 1999. The *lazy* mutation in rice affects a step between statoliths and gravity-induced lateral auxin transport. Plant Biol 1:379–381.

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

Harper RM, Stowe-Evans EL, Luesse DR, Muto H, Tatematsu K, Watahiki MK, Yamamoto K, Liscum E. 2000. The *nph4* locus encodes the auxin response factor ARF7, a conditional regulator of differential growth in aerial Arabidopsis tissue. Plant Cell 12:757–770.

Hasenstein K, Blancaflor E, Lee J. 1999. The microtubule cytoskeleton does not integrate auxin transport and gravitropism in maize roots. Physiol Plant 105:729–738. DOI: 10.1034/j.1399-3054.1999.105418.x

Himmelspach R, Nick P. 2001. Gravitropic microtubule reorientation can be uncoupled from growth. Planta 212:184–189.

Himmelspach R, Wymer CL, Lloyd CW, Nick P. 1999. Gravity-induced reorientation of cortical microtubules observed in vivo. Plant J 18:449–453. DOI: 10.1046/j.1365-313X.1999.00467.x

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.

Iino M. 1991. Mediation of tropisms by lateral translocation of endogenous indole-3-acetic acid in maize coleoptiles. Plant Cell Environ 14:279–286.

Iino M. 1995. Gravitropism and phototropism of maize coleoptiles: evaluation of the Cholodny-Went theory through effects of auxin application and decapitation. Plant Cell Physiol 36:361–367.

Ishikawa H, Evans ML. 1992. Induction of curvature in maize roots by calcium or by thigmostimulation. Plant Physiol 100:762–768.

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.

Jacobs M, Rubery PH. 1988. Naturally occurring auxin transport regulators. Science 241:346–349.

Johannes E, Collings D, Rink J, Allen N. 2001. Cytoplasmic pH dynamics in *zea mays* pulvinal cells induced by gravity vector changes. Plant Physiol : (in press)

Jones A. 1992. What remains of the Cholodny-Went theory? Asymmetric redistribution of auxin need only occur over the distance of one cell width. Plant Cell Environ 15:775–776.

Jones AM. 1990. Location of transported auxin in etiolated maize shoots using 5-azidoindole-3-acetic acid. Plant Physiol 93:1154–1161.

Jones AM. 1998. Auxin transport: down and out and up again. Science 282:2201–2203.

Kamada M, Fujii N. 2000. Control of gravimorphogenesis by auxin: accumulation pattern of *CS-IAA1* mRNA in cucumber seedlings grown in space and on the ground. Planta 211:493–501.

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

Kaufman P, Wu L, Brock T, Kim D. 1995. Hormones and the orientation of growth. In: Davies P, editor. Plant hormones: physiology, biochemistry, and molecular biology. Kluwer Dordrecht, The Netherlands: Academic Publishers. p 547–571.

Kiss J. 2000. Mechanisms of the early phases of plant gravitropism. Crit Rev Plant Sci 19:551–573. DOI: 10.1016/S0735-2689(01)80008-3

Larkin P, Bison J, Mathesius U, Weinman J, Gartner E, Hall E, Tanner G, Rolfe B, Djordjevic M. 1996. Trangenic white clover. Studies with the auxin -responsive promoter, *GH3*, in root gravitropism and lateral root development. Transgen Res 5:325–335.

Legue V, Blancaflor E, Wymer C, Perbal G, Fantin D, Gilroy S. 1997. Cytoplasmic free Ca2+ in arabidopsis roots changes in response to touch but not gravity. Plant Physiol 114:789–800.

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.

Liscum E, Stowe-Evans EL. 2000. Phototropism: A "simple" physiological response modulated by multiple interacting photosensory-response pathways. Photochem Photobiol 72:273–282.

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

Long J, Zhao W, Rashotte A, Muday G, Huber S. 2001. Gravity-stimulated changes in free auxin and invertase gene expression in maize pulvinal cells. Plant Physiol in press

Lu C, Fedoroff N. 2000. A mutation in the arabidopsis *hyl1* gene encoding a dsRNA binding protein affects responses to abscisic acid, auxin, and cytokinin. Plant Cell 12:2351–2366. DOI: 10.1105/tpc.12.12.2351

Lu YT, Feldman LJ. 1997. Light-regulated root gravitropism: a role for, and characterization of, a calcium/calmodulin-dependent protein kinase homolog. Planta 203:S91–S97.

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. DOI: 10.1093/emboj/18.8.2066

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

McClure B, Guilfoyle T. 1989. Rapid redistribution of auxin-regulated rnas during gravitropism. Science 243:91–93.

Morris D. 2000. Transmembrane auxin carrier systems- dynamic regulators of polar auxin transport. Plant Growth Reg 32:161–172.

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. DOI: 10.1007/s004250050363

Morris DA, Rubery PH, Jarman J, Sabater M. 1991. Effects of inhibitors of protein synthesis on transmembrane auxin transport in *Cucurbita pepo* 1. Hypocotyl segments. J. Exper Bot 42:773–783.

Muday G. 2000a. 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. The Netherlands: Kluwer Academic Press, Dordrecht. p 541–556.

Muday G. 2000b. Maintenance of asymmetric cellular localization of an auxin transport protein through interaction with the actin cytoskeleton. J Plant Growth Reg 19:385–396.

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.

Mullen JL, Ishikawa H, Evans ML. 1998. Analysis of changes in relative elemental growth rate patterns in the elongation zone

of arabidopsis roots upon gravistimulation. Planta 206:598–603. DOI: 10.1007/s004250050437

Muller A, Guan C, Galweiler 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. DOI: 10.1093/emboj/17.23.6903

Murphy A, Peer W, Taiz L. 2000. Regulation of auxin transport by aminopeptidases and endogenous flavonoids. Planta 211:315–324. DOI: 10.1007/s004250050003

Murphy A, and Taiz L. 1999. Naphthylphthalamic acid is enzymatically hydrolyzed at the hypocotyl-root transition zone and other tissues of Arabidopsis thaliana seedlings. Plant Physiol Biochem 37:413–430. DOI: 10.1016/S0981-9428(99)80047-3

Nebenfuhr A, White TJ, Lomax TL. 2000. The *diageotropica* mutation alters auxin induction of a subset of the aux/iaa gene family in tomato. Plant Mol Biol 44:73–84. DOI: 10.1023/A:1006437205596

Nick P, Bergfeld R, Schafer E, Schopfer P. 1990. Unilateral reorienation of microtubules at the outer epidermal cell wall during photo and gravitropic curvature of maize coleoptiles and sunflower hypocotyls. Planta 181:162–168.

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 Op 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.

Parry G, Delbarre A, Marchant A, Swarup R, Napier R, Perrot-Rechenmann C, Bennett MJ. 2001. Novel auxin transport inhibitors phenocopy the auxin influx carrier mutation *aux1.* Plant J 25:399–406. DOI: 10.1046/j.1365-313X.2001.00970.x

Peer W, Murphy A, Brown D, Tague B, Muday G, Taiz L. 2001. Flavonoid accumulation patterns of *transparent testa* mutants of Arabidopsis. Plant Physiol 126:536–548. DOI: 10.1104/pp.126.2.536

Perera IY, Heilmann I, Boss WF. 1999. Transient and sustained increases in inositol 1,4,5-trisphosphate precede the differential growth response in gravistimulated maize pulvini. Proc Natl Acad Sci U S A 96:5838–5843. DOI: 10.1073/pnas.96.10.5838

Perera IY, Heilmann I, Chang SC, Boss WF, Kaufman PB. 2001. A role for inositol 1,4,5-trisphosphate in gravitropic signaling and the retention of cold-perceived gravistimulation of oat shoot pulvini. Plant Physiol 125:1499–1507. DOI: 10.1104/pp.125.3.1499

Philippar K, Fuchs I, Luthen H, Hoth S, Bauer CS, Haga K, Thiel G, Ljung K, Sandberg G, Bottger M, Becker D, Hedrich R. 1999. Auxin-induced K(+) channel expression represents an essential step in coleoptile growth and gravitropism. Proc Natl Acad Sci U S A 96:12186–12191. DOI: 10.1073/pnas.96.21.12186

Poupart J, Waddell CS. 2000. The *rib1* mutant is resistant to indole-3-butyric acid, an endogenous auxin in Arabidopsis. Plant Physiol 124:1739–1751.

Rahman A, Ahamed A, Amakawa T, Goto N, Tsurumi S. 2001. Chromosaponin I specifically interacts with *aux1* protein in regulating the gravitropic response of arabidopsis roots. Plant Physiol 125:990–1000. DOI: 10.1104/pp.125.2.990

Rashotte A, DeLong A, Muday G. 2001. Genetic and chemical reductions in protein phosphatase activity alter auxin transport, gravity response and lateral root growth. Plant Cell 13:1683–1697.

Rashotte AM, Brady SR, Reed RC, Ante SJ, Muday GK. 2000. Basipetal auxin transport is required for gravitropism in roots of Arabidopsis. Plant Physiol 122:481–490.

Rayle DL, Cleland RE. 1992. The acid growth theory of auxin-induced cell elongation is alive and well. Plant Physiol 99:1271–1274.

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.

Rice MS, Lomax TL. 2000. The auxin-resistant *diageotropica* mutant of tomato responds to gravity via an auxin-mediated pathway. Planta 210:906–913.

Rubery PH. 1990. Phytotropins: receptors and endogenous ligands. Symp Soc Exp Biol 44:119–146.

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

Ruegger M, Dewey E, Gray WM, Hobbie L, Turner J, Estelle M. 1998. The *tir1* protein of Arabidopsis functions in auxin response and is related to human *skp2* and yeast *grr1p.* Genes Dev 12:198–207.

Ruegger M, Dewey E, Hobbie L, Brown D, Bernasconi P, Turner J, Muday G, Estelle M. 1997. Reduced naphthylphthalamic acid binding in the *tir3* mutant of Arabidopsis is associated with a reduction in polar auxin transport and diverse morphological defects. Plant Cell 9:745–757.

Ruppel NJ, Hangarter RP, Kiss JZ. 2001. Red-light-induced positive phototropism in Arabidopsis roots. Planta 212:424–430.

Sack FD. 1991. Plant gravity sensing. Int Rev Cytol 127:193–254.

Sakai T, Kagawa T, Kasahara M, Swartz TE, Christie JM, Briggs WR, Wada M, Okada K. 2001. Arabidopsis *nph1* and *npl1:* blue light receptors that mediate both phototropism and chloroplast relocation. Proc Natl Acad Sci U S A 98:6969–6974. DOI: 10.1073/pnas.101137598

Sakoda M, Hasegawa K. 1989. Phototropism in hypocotyls of radish. Vi. No exchange of endogenous indole-3-acetic acid between peripheral and central cell layers during first and second positive phototropic curvatures. Phys Plant 76:240–242.

Salisbury FB, Gillespie L, Rorabaugh P. 1988. Gravitropism in higher plant shoots V: changing sensitivity to auxin. Plant Physiol 88:1186–1194.

Scott AC, Allen NS. 1999. Changes in cytosolic ph within arabidopsis root columella cells play a key role in the early signaling pathway for root gravitropism. Plant Physiol 121:1291–1298.

Sedbrook JC, Chen R, Masson PH. 1999. *Arg 1* (altered response to gravity) encodes a DNAJ-like protein that potentially interacts with the cytoskeleton. Proc Natl Acad Sci USA 96:1140–1145. DOI: 10.1073/pnas.96.3.1140

Sievers A, Buchen B, Hodick D. 1996. Gravity sensing in tip-growing cells. Trends Plant Sci 1:273–279. DOI: 10.1016/1360-1385(96)10028-5

Sinclair W, Oliver I, Maher P, Trewavas A. 1996. The role of calmodulin in the gravitropic response of the *Arabidopsis thaliana agr3* mutant. Planta 199:343–351.

Sinclair W, Trewavas A. 1997. Calcium in gravitropism. A re-examination. Planta 203:S85–S90.

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, Galweiler L, Palme K, Jurgens G. 1999. Coordinated polar localization of auxin effiux carrier *pin1* by *gnom arf gef.* Science 286:316–318. DOI: 10.1126/science.286.5438.316

Stowe-Evans E, Leusse D, Liscum E. 2001. The enhancement of phototropin-induced phototropic curvature in Arabidopsis occurs via a photoreversible phytochrome a-dependent modulation of auxin responsiveness. Plant Physiol 126:826–834. DOI: 10.1104/pp.126.2.826

Stowe-Evans EL, Harper RM, Motchoulski AV, Liscum E. 1998. Nph4, a conditional modulator of auxin-dependent differential growth responses in arabidopsis. Plant Physiol 118:1265–1275.

Takahashi H. 1997. Hydrotropism: the current state of our knowledge. J Plant Res 110:163–169.

Takesue K, Shibaoka H. 1998. The cyclic reorientation of cortical microtubules in epidermal cells of azuki bean epicotyls: the role of actin filaments in the progression of the cycle. Planta 205:539–546. DOI: 10.1007/s004250050353

Tasaka M, Kato T, Fukaki H. 1999. The endodermis and shoot gravitropism. Trends Plant Sci 4:103–107. DOI: 10.1016/S1360-1385(99)01376-X

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

Tsurumi S, Ohwaki Y. 1978. Transport of ^{14}C-labeled indoleacetic acid in *Vicia* root segments. Plant Cell Physiol 19:1195–1206.

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.

Vitha S, Zhao L, Sack FD. 2000. Interaction of root gravitropism and phototropism in Arabidopsis wild-type and starchless mutants. Plant Physiol 122:453–462.

Wang Q, Nick P. 1998. The auxin response of actin is altered in the rice mutant *yin-yang.* Protoplasma 204:22–33.

Watahiki MK, Yamamoto KT. 1997. The *massugul* mutation of arabidopsis identified with failure of auxin- induced growth curvature of hypocotyl confers auxin insensitivity to hypocotyl and leaf. Plant Physiol 115:419–426.

Wayne R, Staves MP, Leopold AC. 1990. Gravity-dependent polarity of cytoplasmic streaming in *Nitellopsis.* Protoplasma 155:43–57.

Wilkinson S, Morris DA. 1994. Targeting of auxin carriers to the plasma membrane: effects of monensin on transmembrane auxin transport in *cucurbita pepo* 1. Tissue. Planta 193:194–202.

Wolverton C, Mullen J, Isikawa H, Evans M. 2000. Two distinct regions of response drive differential growth in vigna root electrotropism. Plant Cell Environ 23:1275–1280. DOI: 10.1046/j.1365-3040.2000.00629.x

Wyatt SE, Rashotte AM, Muday GK, Robertson D, Mutations in the *gps* loci in arabidopsis disrupt the perception and/or signal transduction of gravitropic stimuli. Personal communication.

Yamauchi Y, Fukaki H, Fujisawa H, Tasaka M. 1997. Mutations in the *sgr4,sgr5* and *sgr6* loci of *Arabidopsis thaliana* alter the shoot gravitropism. Plant Cell Physiol 38:530–535.

Yang RL, Evans ML, Moore R. 1990. Microsurgical removal of epidermal and cortical cells: evidence that the gravitropic signal moves through the outer cell layers in primary roots of maize. Planta 180:530–536.

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.

Zhong R, Ye ZH. 2001. Alteration of auxin polar transport in the arabidopsis *ifl1* mutants. Plant Physiol 126:549–563. DOI: 10.1104/pp.126.2.549

Geldner N, Friml J, Stierhof YD, Jurgens G, Palme K. 2001. Auxin transport inhibitors block PIN1 cycling and vesicle trafficking. Nature 413:425–428.

Estelle M. 2001. Plant hormones: transporters on the move. Nature 413:374–375.