Plant Molecular Biology **49:** 273–284, 2002.
Perrot-Rechenmann and Hagen (Eds.), Auxin Molecular Biology.

Polar auxin transport – old questions and new concepts?

Jiří Friml[1,2,*] and Klaus Palme[3]
[1]*Zentrum für Molekularbiologie der Pflanzen, Universität Tübingen, Auf der Morgenstelle 3, 72076 Tübingen, Germany (*author for correspondence; e-mail jiri.friml@zmbp.uni-tuebingen.de);* [2]*Department of Biochemistry, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic (e-mail friml@chemi.muni.cz);* [3]*Max-Delbrück-Laboratorium in der Max-Planck-Gesellschaft, Carl-von-Linné-Weg 10, 50829 Köln, Germany*

Received 13 September 2001; accepted 22 October 2001

Key words: auxin, polar auxin transport

Abstract

Polar auxin transport controls multiple aspects of plant development including differential growth, embryo and root patterning and vascular tissue differentiation. Identification of proteins involved in this process and availability of new tools enabling 'visualization' of auxin and auxin routes *in planta* largely contributed to the significant progress that has recently been made. New data support classical concepts, but several recent findings are likely to challenge our view on the mechanism of auxin transport. The aim of this review is to provide a comprehensive overview of the polar auxin transport field. It starts with classical models resulting from physiological studies, describes the genetic contributions and discusses the molecular basis of auxin influx and efflux. Finally, selected questions are presented in the context of developmental biology, integrating available data from different fields.

Abbreviations: AEI, auxin efflux inhibitors; *agr*, agravitropic; ARF, auxin response factor; ARF GEF, guanine-nucleotide exchange factor on ADP-ribosylation factor G protein; BFA, Brefeldin A; *eir*, ethylene-insensitive root; IAA, indole-3-acetic acid; 1-NAA, 1-naphtylacetic acid; NPA, 1-*N*-naphthylphthalamic acid; PAT, polar auxin transport; *rcn1*, roots curl in NPA; TIBA, 2,3,5′-triiodobenzoic acid; *tir*, transport inhibitor response

Historical concepts

Polar auxin transport represents a unique process specific to plants and the phytohormone auxin. Already in the 19th century classical experiments studying the phototropism of canary grass coleoptiles indicated the existence of a transmissible signal, which was later identified and termed auxin (Darwin and Darwin, 1881). Further research in this field led to the formulation of the Cholodny-Went hypothesis, which proposed that differential auxin distribution represents the mechanism for tropic growth (summarized in Went, 1974; Firn *et al.*, 2000). Auxin transport experiments using chemical inhibitors revealed the physiological importance of auxin transport and led to the formulation of the chemiosmotic model that explains auxin transport at the cellular level through the action of specific influx and efflux carriers (Rubery and Sheldrake, 1974; Raven, 1975). Joint efforts of genetics and molecular biology resulted in identification of genes encoding putative auxin influx and efflux carrier proteins represented by *AUX* and *PIN* gene families respectively (Palme and Gälweiler, 2000). Although direct biochemical evidence establishing their function as carriers is still lacking, it is undisputable that AUX and PIN proteins participate in the auxin transport process. Today the availability of new tools for localization of these proteins (Palme and Gälweiler, 2000; Friml *et al.*, 2001b; Swarup *et al.*, 2001) and *in situ* monitoring of auxin accumulation (Sabatini *et al.*, 1999) as well as availability of mutants impaired in auxin transport (Palme and Gälweiler, 2000; Friml *et al.*, 2001b) enable us to give the old physiological theories new molecular meaning and precisely address the role of auxin transport in many developmental processes. So far these studies largely support

the widely accepted classical concepts and give a fresh input to old discussions about auxin as a plant morphogen (Sabatini *et al.*, 1999; Friml *et al.*, 2001b). However recent data suggest that auxin efflux carriers may not solely act at the plasma membrane. Based on these data a new view on the mode of auxin transport inhibitors action is proposed (Geldner *et al.*, 2001). These findings are likely to change our long-held views about auxin transport and, therefore, further advances in this field are eagerly awaited.

Physiology of auxin transport

Auxin is thought to be synthesized in plants locally, in young growing regions, predominantly in the shoot apex, young leaves and developing seeds (Normanly *et al.*, 1991; Ljung *et al.*, 2001), but it seems that almost any plant tissue can at certain times be responsive for auxin (Davies, 1995). Already in the 1920s, Cholodny and Went were independently trying to hypothesize how auxin moves from the apex into the elongation zone (Went, 1974).

Two main pathways describe the transport of auxin, a fast, non-directional transport in the phloem and a slower, directional, so-called polar auxin transport (PAT) in various tissues. The evidence for the existence of phloem transport was established through experiments with radioactively labelled auxin (Morris and Thomas, 1978). This transport occurs in both basipetal and acropetal directions, proceeds relatively fast (5–20 cm/h) and seems to correlate well with the transport of assimilates and inactive auxin conjugates (Nowacki and Bandurski, 1980). Direct auxin analysis revealed physiological relevant amounts of free indole-3-acetic acids (IAA) within the phloem exudate (Baker, 2000). Experiments in pea showed that the labeled auxin transported within the phloem was later detected in the PAT system indicating that both transport pathways may be linked (Cambridge and Morris, 1996).

In contrast to phloem transport, PAT is specific for active free auxins, occurs in a cell-to-cell manner and has a strictly unidirectional character. The main PAT stream runs from the apex basipetally with a velocity of 5–20 mm/h towards the base of the plant (Lomax *et al.*, 1995). Using radioactively labelled auxin this kind of transport was mainly detected in the cambium and adjacent, partially differentiated xylem elements (Morris and Thomas, 1978). In roots the auxin stream continues acropetally towards the root tip, where part of the auxin is redirected backwards and transported basipetally through the root epidermis to the elongation zone (Rashotte *et al.*, 2000). In contrast, PAT in shoots occurs in lateral direction (Morris and Thomas, 1978). Auxin transport assays also revealed that PAT requires energy, is saturable and sensitive to protein synthesis inhibitors. These results suggested the existence of specific auxin transport proteins and led in the middle of the 1970s to the formulation of a coherent model for auxin transport, termed the chemiosmotic hypothesis (Rubery and Sheldrake, 1974; Raven, 1975). In the relatively acidic environment of the cell wall (pH around 5.5) about 15% of IAA exists in its protonated form (IAAH). This noncharged, lipophilic molecule passes easily through the plasma membrane by diffusion. In the more basic cytoplasm (pH around 7) IAAH dissociates and hence the resulting IAA^-anion is 'trapped' in the cell due to its poor membrane permeability. Therefore, a specific efflux carrier was postulated and the polarity of the auxin flux was explained by its asymmetric distribution in cells. In addition, the existence of specific auxin influx carriers was hypothesized (Goldsmith, 1977) and later saturable auxin influx, probably working as an $IAA^-/2H^+$ co-transporter, was physiologically demonstrated (Lomax *et al.*, 1985; Benning, 1986). Auxin influx and efflux pathways can be physiologically distinguished using auxin efflux inhibitors (AEI). These pharmacological tools arose from correlative exploration of structure-activity profiles of chemicals with auxin-like activity (Katekar and Geissler, 1977) and were demonstrated to inhibit efflux of auxin from cells and hypocotyl segments (reviewed in Rubery, 1990). Most widely recognized AEI such as 1-*N*-naphthylphthalamic acid (NPA) share a benzoic acid ortho-linked with an aromatic ring system. However other very effective inhibitors such as 2,3,5-triiodobenzoic acid (TIBA) do not share this structural pattern. In order to explain the mechanism of inhibition of auxin efflux by AEI, the existence of an NPA-binding protein forming part of the auxin efflux carrier complex was postulated. Additional experiments with auxin efflux and NPA binding studies in the presence of inhibitors of protein synthesis suggested the existence of a third unstable component of the auxin efflux carrier complex likely coupling the NPA-binding protein to auxin transport protein (Morris *et al.*, 1991). Attempts to localize NPA-binding protein within the cell through NPA binding studies led to controversial results favouring either association with cytoskeleton (Cox and Mudday, 1994) or inte-

gral membrane localization (Bernasconi *et al.*, 1996). Despite very limited data on the molecular mechanism of AEI function, they have shown to be valuable tools for the establishment of the role of the auxin efflux carrier in plant development. Due to the lack of similar inhibitors of auxin influx the characterization of the corresponding protein complex and elucidation of the role of auxin influx in plant development was retarded. Nevertheless, the recent isolation of compounds such as 1-naphthoxyacetic acid (1-NOA) and 3-chloro-4-hydroxyphenylacetic acid (CHPAA) specifically inhibiting auxin uptake in tobacco culture cells has re-established the balance (Imhoff *et al.*, 2000).

Genetic and molecular analysis of auxin transport

The biochemical attempts to isolate the components of auxin influx and efflux carrier complexes and other proteins involved in PAT have been so far not fully successful. The main contributions concerning their identity come from the genetic field, especially from the analysis of *Arabidopsis* mutants. Different screening strategies have been successfully applied to identify mutants affected in PAT. Some mutants have been selected on the basis of abnormal responses to auxin transport inhibitors or were identified fortuitously in screens for developmental alterations and only later the connection to PAT was discovered. A mutant called *rcn1* was isolated, whose roots curl in the presence of NPA in contrast to straight root growth in wild type (Garbers *et al.*, 1996). This mutant shows a reduction in root and hypocotyl elongation and is also defective for apical hook formation. The *RCN1* gene was cloned and shown to encode a protein phosphatase II A subunit. *RCN1* may control the level of phosphorylation and thereby the activity of a component involved in PAT (Garbers *et al.*, 1996). This hypothesis was corroborated by recent findings that the *rcn* mutant displays enhanced basipetal auxin transport, a phenotype feature, which was also observed in plants treated with the phosphatase inhibitor cantharidin (Rashotte *et al.*, 2001). The *pinoid* (*pid*) mutants display defects in the formation of flowers and cotyledons resembling plants grown on AEI. Moreover, auxin transport in *pid* inflorescences is reduced (Okada *et al.*, 1991). The *PID* gene has been recently cloned and shown to encode a serine-threonine protein kinase, which was proposed to act as a negative regulator of auxin signaling (Christensen *et al.*, 2000). However, another analyses demonstrate that PID action is sensitive to AEI thus favouring the hypothesis that PID functions as a positive regulator of auxin transport (Benjamins *et al.*, 2001). Thus analysis of both RCN and PID highlights a role of phosphorylation in the regulation of PAT. The *tir* (transport inhibitor response) mutants were isolated on their ability to allow root elongation in the presence of AEI (Ruegger *et al.*, 1997). The mutant called *tir3* displays a variety of morphological defects including reduced elongation of root and inflorescences, decreased apical dominance and reduced lateral root formation. Both auxin transport and NPA-binding activity are reduced in *tir3* mutants (Ruegger *et al.*, 1997), suggesting that the *TIR3* gene may encode the NPA-binding protein or some closely related protein (Hobbie, 1998). The corresponding gene designated as *BIG* has been recently cloned (Gil *et al.*, 2001) and shown to encode a protein with several putative Zn-finger domains homologous to the *Drosophila* Calossin (CalO)/Pushover protein. A defect in CalO interferes with neurotransmitter release in *Drosophila*. The *big* mutation interferes with an effect of auxin efflux inhibition on putative auxin efflux barrier AtPIN1 cycling (Gil *et al.*, 2001) supporting the function of *BIG* in vesicle trafficking, although the mechanism of action has not yet been not clarified.

AUX1 protein: an influx carrier?

Another mutant called *aux1*, which confers a root agravitropic and auxin-resistant phenotype, was instructive for identification of a gene possibly encoding an auxin influx carrier (Bennett *et al.*, 1996). The *aux1* phenotype is consistent with a defect in auxin influx, but similar phenotypes have been observed also in mutants defective in auxin response (Lincoln *et al.*, 1990). The *AUX1* gene encodes a 485 amino acid protein sharing significant similarity with plant amino acid permeases favouring the role for AUX1 in the uptake of the tryptophan-like IAA (Bennett *et al.*, 1996). Despite the fact that final biochemical proof of AUX1 function as an auxin uptake carrier is still lacking, several lines of evidence strongly support the involvement of AUX1 in auxin influx. The strongest support came from a detailed analysis of the *aux1* phenotype. It has been demonstrated that the membrane permeable 1-NAA rescues the *aux1* root agravitropic phenotype much more efficiently than membrane less permeable IAA or 2,4-D and that this rescue coincides with restoration of basipetal auxin transport (Yamamoto and Yamamoto, 1998; Marchant *et al.*, 1999). More-

over, this phenotype including its specific NAA rescue can be mimicked by growing seedlings on recently isolated inhibitors of auxin influx (Parry *et al.*, 2001). The most direct support that AUX1 participates in auxin influx came from the comparison of auxin transport properties of *aux1* and wild-type roots. Uptake assays using radioactively labelled auxins and auxin analogues revealed that *aux1* roots accumulated significantly less 2,4-D than wild-type roots (Marchant *et al.*, 1999). Interestingly, such a difference was not found when the membrane-permeable1-NAA or the IAA-like amino acid tryptophan were assayed. Recently the AUX1 protein was localized within *Arabidopsis* root tissue by an epitope tagging approach (Swarup *et al.*, 2001). The AUX1 protein was detected in a remarkable pattern in a subset of stele, columella, lateral root cap and epidermal cells exclusively in the root tips (Figure 1). Disruption of AUX1 causes changes in cell-specific auxin accumulation associated with tissues mediating basipetal auxin transport (Swarup *et al.*, 2001; Rashotte *et al.*, 2001). However, *aux1* mutants are also defective in auxin supply to the root tip, since mutant root tips contain less free auxin than those of wild type. This paradox taken together with localization of AUX1 at the upper side of protophloem cells suggest a role of AUX1 protein in unloading of the bulk flow via the protophloem to the root apical meristem (Swarup *et al.*, 2001). Thus AUX1 would appear to provide the first molecular connection between polar and non-polar auxin transport routes.

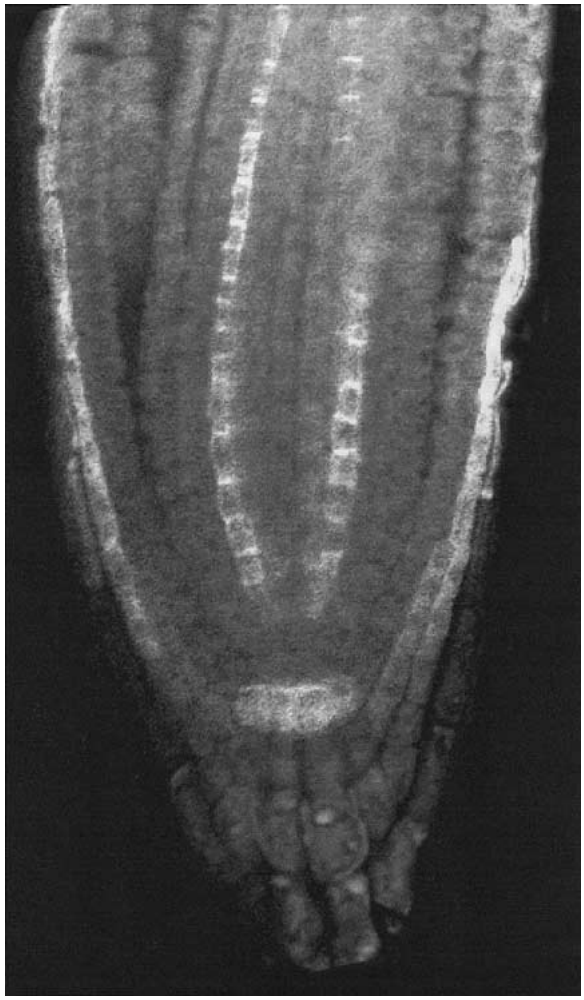

Figure 1. The AUX1 protein localizes to the apical surface of protophloem cells, to the columella and to the lateral root cap.

PIN proteins: efflux carriers?

The knitting needle-like *pin1* mutant phenotype strongly resembles plants treated with inhibitors of auxin efflux. In addition, this mutant displays a strong reduction in basipetal auxin transport (Okada *et al.*, 1991). The *AtPIN1* gene was cloned by transposon tagging and found to encode a 622 amino acid protein with up to 12 putative transmembrane segments. AtPIN1 similarity to a group of transporters from bacteria supports a role of this protein as an auxin efflux carrier (Gälweiler *et al.*, 1998). Alternatively and equally likely on the basis of currently available genetic evidence, it could act as a regulator of auxin transport. Almost simultaneously a homologous gene was found by several laboratories, *AtPIN2/EIR1/AGR1* (Chen *et al.*, 1998; Luschnig *et al.*, 1998; Müller *et al.*, 1998; Utsuno *et al.*, 1998), and cloning and analysis of additional sequences (*AtPIN3* and *AtPIN4*) followed (Friml *et al.*, 2001a, b). In total, the *Arabidopis PIN* gene family consists of eight members and homologous genes were found in other plant species such as maize, rice and soybean. As for the role of AUX1 in auxin influx, the proposed function for PIN proteins as efflux carriers has not been biochemically demonstrated to date. However, there are several lines of evidence strongly supporting a role of PIN proteins in auxin transport and particularly in auxin efflux.

AtPIN protein sequences and topology suggest transport function

The AtPIN proteins share more than 70% similarity and have an identical topology – two highly hydrophobic domains with five to six transmembrane segments

linked by a hydrophilic region. Transporters of the major facilitator class display similar topology. Moreover, PIN proteins were demonstrated to share limited sequence similarity with prokaryotic and eukaryotic transporters (Palme and Gälweiler, 1999, Müller *et al.*, 1998; Luschnig *et al.*, 1998; Chen *et al.*, 1998; Utsuno *et al.*, 1998) supporting a proposed transport function for PIN proteins.

Yeast over-expressing AtPIN2 are resistant to fluoroindole

To date, the only experimental system used for to address AtPIN transport activity were yeast cells carrying a mutation in the *GEF1* gene resulting in altered ion homeostasis (Gaxiola *et al.*, 1998; Luschnig *et al.,* 1998). Yeast cells defective in this gene and over-expressing AtPIN2 (in these experiments designated EIR1 and AGR1) showed enhanced resistance to the yeast toxin fluoroindole, a substance with some albeit limited structural similarity to auxin (Luschnig *et al.*, 1998). Other experiments demonstrated that AtPIN2 over-expressing yeast retain less radioactively labelled auxin than control yeast (Chen *et al.*, 1998). The decreased accumulation of labelled auxins or toxic auxin analogues may indicate an auxin efflux function for AtPIN2 protein in yeast. Nevertheless, direct measurements of auxin efflux instead of auxin retention have not been demonstrated, leaving this issue unsolved.

AtPIN proteins are localized in a polar manner in auxin transport competent cells

The classical concept of chemiosmotic hypothesis predicted that auxin efflux carriers adopt a polar localization (Rubery and Sheldrake, 1974; Raven, 1975). Such a remarkable localization in accordance with the known direction of auxin flux has been demonstrated for several AtPIN proteins in PAT-competent cells. AtPIN1 protein is localized at the lower side of elongated parenchymatous xylem and cambial cells of *Arabidopsis* inflorescence axes in accordance with the basipetal direction of PAT (Gälweiler *et al.*, 1998; Palme and Gälweiler, 2000). In contrast, the AtPIN2 protein was polarly localized at the upper side of the lateral root cap and epidermis cells (Müller *et al.*, 1998). The AtPIN3 protein localizes predominantly to the lateral side of shoot endodermis cells (Friml *et al.*, 2002a) and the polar localization of AtPIN4 in root directs towards columella initials, the site of auxin accumulation in the root apex (Sabatini *et al.*, 1999; Friml *et al.*, 2002b).

Atpin mutants are defective in PAT

One of the strongest arguments for the involvement of PIN proteins in auxin transport is a reduction of PAT in *Atpin* mutants, which directly correlates with loss of AtPIN expression in corresponding tissue. This was demonstrated for basipetal auxin transport in stem of *Atpin1* mutant or in root of *Atpin2* mutant (Okada *et al.*, 1991; Rashotte *et al.*, 2000).

Disruption of AtPIN function cause changes in cell-specific auxin accumulation

Auxin accumulation has been indirectly monitored by the activity of an auxin-responsive construct (e.g. *DR5::GUS*; Sabatini *et al.*, 1999), which seems to correlate very well with direct free IAA measurements (Casimiro *et al.*, 2001; Friml *et al.*, 2002b). Using this approach changes in cell-specific auxin accumulation were found in several *Atpin* mutants which correlated with loss of AtPIN expression in corresponding tissues. These changes were demonstrated for *Atpin2* (in these experiments called *eir1*) mutant roots after changes in gravistimulation (Luschnig *et al.*, 1998) and for *Atpin4* mutant roots and embryos where the defects in establishment and maintenance of the characteristic auxin response maximum were observed (Friml *et al.,* 2002b). The use of radioactively labelled auxins enables more direct determination of auxin content than the visualization by auxin-responsive promotors, but does not provide the cellular resolution. These experiments in root tips of wild type and *Atpin2* (in these experiments called *agr1*) mutants revealed that with radioactive IAA-preloaded *Atpin2* root tips retain more radioactivity than similarly treated wild-type roots (Chen *et al.*, 1998).

Atpin mutants are hypersensitive to 1-NAA

Loss of auxin efflux carrier activity would be predicted to result in an accumulation of its substrate 1-NAA in plant cells, leading to greater sensitivity towards this auxin analogue. Indeed, root growth assays have revealed that several *Atpin2* mutant alleles exhibit an 1-NAA-hypersensitive phenotype, consistent with our predictions (Müller *et al.*, 1998; Parry and Bennett, personal communication).

Phenotypes of Atpin mutants can be phenocopied by auxin efflux inhibitors

Most of the defects observed in *Atpin* mutants are in processes known to be regulated by PAT and can be phenocopied by AEI. These include:

– the *Atpin1* aerial phenotype with defects in stem vasculature (Gälweiler *et al.*, 1998);
– the affected root and shoot gravitropism in *Atpin2* and *Atpin3* mutants, respectively (Maher and Martindale, 1980, Friml *et al.*, 2002a);
– the defect in hypocotyl and root elongation in light, in apical hook opening and in lateral root initiation, which have been reported for *Atpin3* mutants (our unpublished data);
– the *Atpin4* root meristem pattern aberrations, which can be also found in seedlings germinated on low concentrations of AEI (Friml *et al.,* 2002b).

The data accumulated so far, especially from *Atpin* mutants analyses, provides an extensive body of evidence to argue that AtPIN proteins are involved in some important aspects of auxin transport. However, the central question still remains whether PIN proteins represent either transport or a regulatory components. To answer this question, auxin transport assays have to be developed to establish directly carrier functions of different PIN proteins and to determine their substrate specificities, affinities and kinetic properties.

PIN protein dynamics - turning over the concept

To date the identification of polarly localized PIN proteins as putative efflux carriers and the analysis of corresponding knockout mutants appear to agree with the chemiosmotic hypothesis (Rubery and Sheldrake, 1974; Raven, 1975). However, recent studies focused on cell biological requirements of the polar localization of AtPIN1 protein revealed surprising facts, which are difficult to reconcile with this model. These studies even question the specificity of AEI, which represents one of the most valuable tools to study PAT which have been developed in the past decades. Previous studies have revealed that interference with vesicle trafficking either genetically by disrupting the *GNOM* gene or chemically by Brefeldin A (BFA), a fungal toxin, interferes with correct plasma membrane localization of the AtPIN1 protein (Steinmann *et al.*, 1999). More detailed studies in *Arabidopsis* roots have shown that in the presence of BFA the AtPIN1 protein is rapidly and reversibly internalized from the plasma membrane into the endosomal compartment (Figure 2; Geldner *et al.*, 2001). This also occurred in the presence of a protein synthesis inhibitor thereby demonstrating that internalized AtPIN1 originated from the plasma membrane and that AtPIN1 is rapidly cycling between an endosomal compartment and the plasma membrane. The combinative treatment with BFA and Cytochalasin D or Latrunculin B, which are known to disrupt actin, led to the inhibition of this cycling, suggesting that AtPIN1 vesicles are transported along the actin cytoskeleton. The analysis of effects of AEI on AtPIN1 localization revealed that they leave AtPIN1 at the plasma membrane, but block AtPIN1 cycling. Surprisingly, AEI block also the cycling of other functionally unrelated membrane proteins. This suggested a much more general mode for AEI, namely inhibition of membrane protein trafficking.

It is reasonable to assume that the rapidly cycling AtPIN proteins are components very much sensitive to trafficking inhibition. This taken together with importance of AtPIN-dependent PAT in plant development would explain seemingly specific physiological effects of AEI. Most interestingly, these studies show that the very rapid cycling of the AtPIN1 protein seems to be an essential part of the PAT process and AtPIN1 function, since vesicle trafficking inhibitor BFA is also known to rapidly interfere with auxin efflux and could phenocopy the effect of AEI (Morris and Robinson 1998; Delbarre *et al.*, 1998; Geldner *et al.*, 2001). This surprising finding can be hardly incorporated in the old static models in which influx and efflux carrier complexes sit at the plasma membrane and mediate their transport function. The remaining crucial question is the functional relevance of cycling? Different scenarios can be conceived: (1) a high turnover of auxin efflux complexes would provide important flexibility for rapid changes in polarity of plasma membrane localization and thereby for redirection of an auxin flux; (2) if the hypothesis proposed by Hertel would be true and a component of auxin efflux has a dual receptor/transporter function as was proposed for sugar carriers (Lalonde *et al.*, 1999), the cycling might be part of signal transduction and receptor regeneration, as is known for other kinds of receptors (Knutson, 1991); (3) the most exciting scenario would be that the vesicle trafficking itself is part of the auxin transport machinery and that, in analogy with neurotransmitter release, auxin would be a vesicle cargo, released from cells by polar exocytosis. Regardless whether one or more of these scenarios are true, uncovering of cell mechanisms controlling the subcellular dynamics of the auxin carriers seems to be central for our understanding of auxin transport.

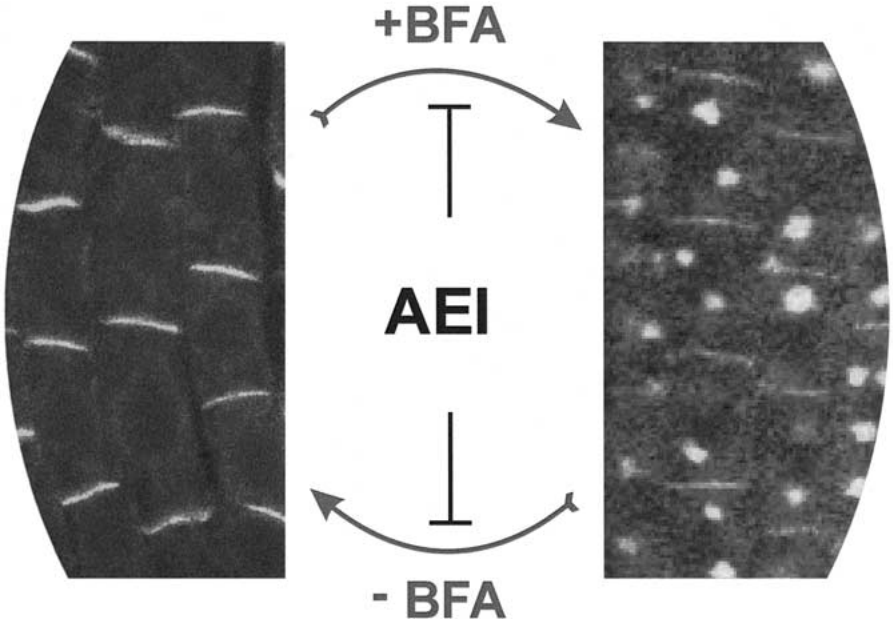

Figure 2. AtPIN1 cycling. The reversible internalization of AtPIN1 label upon Brefeldin A (BFA) treatment suggests that AtPIN1 cycles between the plasma membrane and an endosomal compartment. Polar auxin transport inhibitors (AEI) such as TIBA block AtPIN1 cycling.

The role of polar auxin transport in plant development

The central role of PAT in plant development, especially in tropic growth, vascular development, lateral root initiation, embryo and root patterning as well as apical dominance was initially established by physiological studies using chemical inhibitors of PAT (Lomax *et al.*, 1995). The recent availability of genetic and molecular tools now allows to address more specifically the role of auxin transport in each of these developmental processes.

Vascular tissue development and canalization hypothesis

The manipulation of plants with auxin transport inhibitors drew attention to the role of auxin in vascular development (Camus, 1949). Early grafting experiments demonstrated that buds induced the differentiation of new vascular tissues and the replacement of buds by auxin indicated its importance as the major inductive signal (Wetmore and Rier, 1963). Several reports discussed auxin as a correlative and morphogenetic signal, which influences vascular maturation through a partially self-organizing mechanism (Newcomb *et al.*, 1970; Sachs, 1989). This so-called canalization hypothesis assumes that centres with elevated auxin levels gradually gain more competency for auxin conduction and narrowing auxin flow which finally results in differentiation of vascular strands. Auxin transport inhibition experiments have shown that vascular development in leaves and cotyledons of *Arabidopsis* plants grown on different concentration of NPA was severely affected (Mattsson *et al.*, 1999): secondary veins were not developed and, using higher NPA concentrations, the differentiation of the whole leaf vasculature was completely abolished. These studies together with previous studies on vascular regeneration after wounding (Wetmore and Rier, 1963) seemed to be in very good agreement with the canalization hypothesis and provided a strong link between PAT and vascular development. However, recent results of extensive genetic screens in *Arabidopsis* for mutants defective in vascular patterning led to isolation of seven different loci designated *van1–van7* (Koizumi *et al.*, 2000). Most of these mutations led to fragmentation of otherwise fully differentiated cotyledon vasculature without largely affecting the overall architecture of the vascular network thereby favouring a pre-patterning mechanism for vasculature differentiation (Koizumi *et al.*, 2000). The molecular characterization of these mutants and use of molecular markers for visualization of auxin transport routes and early vascular fate identity will certainly contribute to the

further clarification of the role of auxin canalization versus prepatterning in vascular tissue differentiation.

Differential growth in shoot: a role for AtPIN3-regulated lateral auxin transport?

Differential growth in shoot (gravitropism, phototropism and apical hook formation) have been proposed to result from the asymmetric redistribution of auxin, which subsequently promotes or inhibits cell growth and elongation resulting in bending (Went, 1974). This model gained strong support by visualization of differentially distributed auxin response reporters in gravity-stimulated tobacco shoots (Li *et al.*, 1991), in *Arabidopsis* light and gravity stimulated hypocotyls and newly formed apical hooks (Friml *et al.*, 2002a). In this system PAT inhibitors blocked both the asymmetric distribution of auxin response and differential growth, implicating PAT as the process underlying differential auxin distribution and thereby regulating differential growth (Friml *et al.*, 2002a; Lehman *et al.*, 1996). The lateral auxin transport system has been proposed to facilitate the exchange of auxin between the main basipetal stream in vasculature and peripheral regions of shoot, where control of elongation occurs (Epel *et al.*, 1992). The recent characterization of the putative auxin efflux carrier, AtPIN3, recently provided experimental data for this concept (Friml *et al.*, 2002a). The *Atpin3* mutants display defects in hypocotyl differential growth. Moreover, AtPIN3 expression and localization studies revealed that the AtPIN3 protein is predominantly localized at the lateral side of endodermis cells further supporting the model of lateral auxin transport.

Root gravitropism: an influx and efflux matter

In the root the sites of gravity perception are columella cells and the site of response is the elongation zone, where elevated auxin levels on the lower side lead to the inhibition of growth and thereby downward bending of the root (for an overview, see Chen *et al.*, this issue). The main contribution of PAT to the root gravitropic response was supposed to be the basipetal transport from columella region through epidermis towards the elongation zone. The identification of several root agravitropic mutants (*agr1/eir1/wav6/Atpin2*) in a gene encoding the root-specific putative auxin efflux carrier AtPIN2 supports this hypothesis (Chen *et al.*, 1998; Müller *et al.*, 1998; Luschnig *et al.*, 1998; Utsuno *et al.*, 1998). The AtPIN2 protein was localized to the cortex, epidermis and lateral root cap, predominantly at the upper side of cells, consistent with the model (Figure 3; Müller *et al.*, 1998). The role of AtPIN2 in basipetal auxin transport was further supported by the finding that the auxin response maximum at the lower side of the root after a change of gravity vector is not established and basipetal auxin transport is reduced in *Atpin2* mutants (Luschnig *et al.*, 1998; Rashotte *et al.*, 2000). AUX1 is also involved in both regulation of root gravitropism and auxin transport. *Aux1* mutants display defects in basipetal auxin transport correlating with root agravitropic phenotype (Swarup *et al.*, 2001). The *aux1* mutant phenotype was rationalized by the visualization of AUX1 protein at the plasma membrane of lateral root cap and epidermis cells (Figure 1; Swarup *et al.*, 2001). Thus it seems that both auxin influx and efflux as well as its molecular components are required for root gravitropism. Nonetheless it is still not clear if PAT plays a permissive or regulatory role in root gravitropism and where and how the asymmetry of the auxin distribution is initially established. These questions have recently been addressed by visualization of auxin distribution and auxin carriers in gravistimulated roots. The presence of both putative auxin influx and efflux carriers in columella cells (AUX1, Figure 1, Swarup *et al.*, 2001; AtPIN3,4, Figure 3; Friml *et al.*, 2002a,b) suggests that the asymmetry of auxin distribution may be established there and not during the basipetal transport to the responsive tissue. Moreover, the actin dependent cycling of AtPIN3 and its rapid lateral relocation after gravity stimulation in columella cells provide a likely mechanism for establishment of this asymmetry (Friml *et al.*, 2002a). In this model the sedimentation of statoliths upon gravity stimulation leads to rearrangement of the actin cytoskeleton resulting in relocation of the efflux carrier components. This causes the asymmetric flux of auxin from columella cells and auxin is translocated by basipetal transport to the responsive tissue, where it elicits differential growth.

Root meristem patterning: climbing to the sink

The role of auxin and PAT in roots is not restricted to growth responses. Exogenous manipulation of auxin levels as well as analysis of mutants impaired in auxin signalling demonstrated a role for auxin in regulating the pattern of cell division and differentiation (Kerk *et al.*, 1994; Ruegger *et al.*, 1997) and raised a debate about a possible role of auxin as a morphogen (Sabatini *et al.*, 1999). Similarly to animal morphogens, auxin concentration gradients exist as

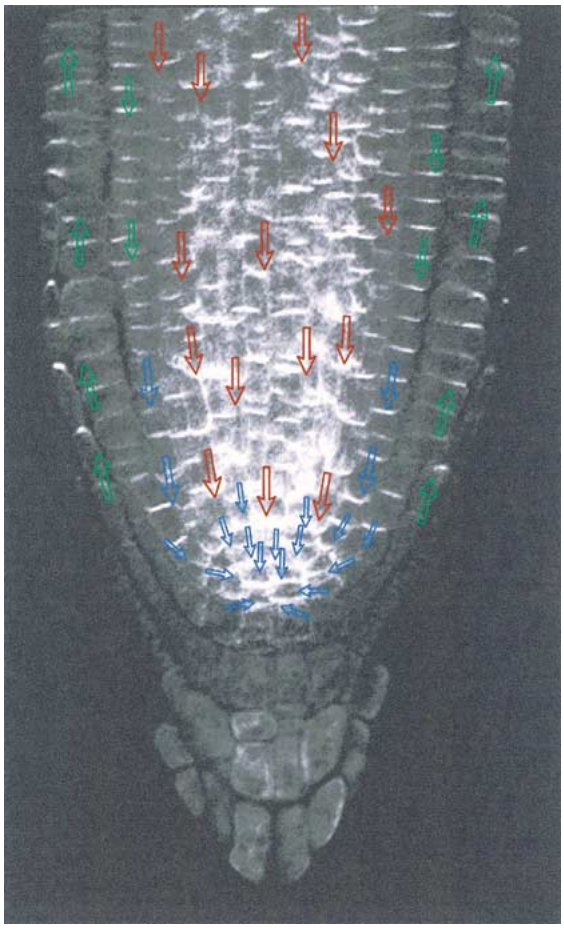

Figure 3. Immunolocalization of AtPIN proteins with presumptive auxin routes in the *Arabidopsis* root tip. AtPIN1 localization suggests auxin flux from apical tissues towards the central root meristem (red arrows), AtPIN4 regulates the focusing of auxin flux into columella establishing the auxin maximum (blue arrows). Part of the auxin is rerouted through outer cell layers backward to the elongation zone regulating root bending (AtPIN2, green arrows).

has been demonstrated by direct auxin measurements in Scots pine (Uggla *et al.*, 1998) as well as by indirect visualization using auxin responsive promoter *DR5* in *Arabidopsis* roots and embryos (Sabatini *et al.*, 1999). In the *Arabidopsis* root the maximum of auxin response was detected in the columella initial cells and first columella layer (Sabatini *et al.*, 1999). Localization studies of the recently characterized AtPIN4 (Figure 3) revealed polar localization pointing towards the same area, suggesting involvement of AtPIN4 in establishment of the auxin gradient with a maximum in columella initials and columella cells. Both chemical inhibition of PAT and the *Atpin4* mutation change the auxin gradient and relocalize its maximum. These changes in auxin gradients were accompanied with various patterning defects and correlated well with changes in cell fate specification thus providing a genetic link between PAT, auxin gradients and patterning. Morphogen gradients known from animal systems are source-driven. Surprisingly, it appears that the data emerging from an analysis of the role of AtPIN4 and PAT in the root meristem can be best integrated into a model involving an active AtPIN4-dependent sink for auxin in the columella region, implying that auxin gradients are sink-driven. According to this model the AtPIN4 protein promotes auxin transport within the central root meristem tissues and actively increases auxin concentration thereby creating maximum in columella cells. The establishing and proper positioning of this maximum seems to be necessary for local down-regulation of auxin, since both NPA-treated roots and *Atpin4* mutant roots display elevated auxin levels in the root tip and fail to canalize exogenously applied auxin properly (Friml *et al.*, 2002b). However, which mechanisms are involved in auxin down-regulation is unclear. Experiments in maize demonstrated high levels of ascorbate oxidase, an enzyme degrading auxin *in vitro*, in the root tip (Kerk *et al.*, 2000). However, there are no data available favouring oxidative decarboxylation as a means for auxin degradation *in vivo*. Another possibility to remove auxin would be active export or passive leaking of the auxin from the root or by pealing off columella cells loaded with auxin. An interesting mechanism would be the continual trafficking of auxin in the apoplast between columella initial and columella cells which is suggested by AtPIN proteins localization. (Friml *et al.*, 2002b). The longer presence of auxin in the oxidative environment of the apoplast would greatly facilitate auxin degradation (G. Sandberg, personal communication).

Embryonic development: establishment of axis

Exogenous manipulations of auxin levels and PAT in carrot callus-derived somatic embryos as well as *in vitro* cultured Indian mustard (*Brassica juncea* L.) zygotic embryos established a role for PAT in the initiation and maintenance of polarized growth in developing embryos (Schiavone *et al.*, 1987; Hadfi *et al.*, 1998). AEI interfered with cotyledon separation highlighting the important role of auxin and its transport in the progression from the radially symmetric to the bilaterally symmetric embryo in analogy to the regulation of radial position of lateral organs in later development (Reinhardt *et al.*, 2000). Genetic studies using

Arabidopsis mutants support a role for PAT in embryo development and suggest a possible role in the establishment of the apical basal axis. The *monopteros* (*mp)* mutant was isolated as an embryo mutant with defects in apical-basal pattern formation (Berleth and Jürgens, 1993), but adult *mp* plants display a strong reduction in PAT (Przemeck *et al.*, 1996). The *MP* gene encodes an auxin-regulated transcription factor from the ARF family raising the possibility that the MP protein could regulate the expression of components of the PAT machinery (Hardtke *et al.*, 1998). Another mutant called *gnom* (*gn*) displays defects strongly resembling AEI-treated embryos (Mayer *et al.*, 1993). The *GN* gene encodes a guanine nucleotide exchange factor of ADP-ribosylation factors (ARF GEF), a protein regulating vesicle trafficking to the plasma membrane (Steinmann *et al.*, 1999). It has been demonstrated that gradual polarity establishment of AtPIN1 localization in *gn* embryos is disturbed suggesting an involvement of GN in proper localization of AtPIN1 (Steinmann *et al.*, 1999). However, in view of recent reports suggesting that vesicle trafficking represents an essential part of PAT (Geldner *et al.*, 2001), *gn* may by disrupting vesicle trafficking directly interfere with this process. Despite the physiologically and genetically well established role of PAT in embryogenesis, little is known about auxin transport routes and auxin accumulation in embryos. Due to technical difficulties especially in tiny *Arabidopsis* embryos, we can deduce these only indirectly from the polarity of AtPIN protein localizations and activity of auxin response reporters. Two *AtPIN* genes, *AtPIN1* and *AtPIN4*, are expressed during embryogenesis and can be found localized in both polar and non-polar fashion (Steinmann *et al.*, 1999; Friml *et al.,* 2002b). AtPIN1 was detected already in the 8-cell stage at the inner cell boundaries. The polarity of the AtPIN1 localization is then gradually established at the globular stage at the basal side of provascular cells, where also AtPIN4 protein is localized. The establishment of AtPIN1 and AtPIN4 polarity in provascular initials coincides with that establishment of a basally positioned *DR5* auxin response maximum (Friml *et al.*, 2002b). At the late globular and triangular stage the polar localization of AtPIN1 is also established in epidermis cells towards the position of future outgrowing cotyledons. During further progression towards the torpedo stage the vascular precursor cells become marked at their basal sides forming a typical Y-shaped pattern. These AtPIN1 and AtPIN4 localization patterns suggest auxin flux towards the quiescent centre, below which an auxin response maximum is formed. The AtPIN4 protein was also detected at the basal side of upper suspensor cells (Friml *et al.*, 2002b), suggesting that auxin homeostasis may be regulated via the suspensor. However, such deduced auxin routes are hypothetical and there are no data available yet on a role of auxin influx carriers during this early stage of development. Thus, the role of PAT in embryogenesis remains still far from clarified. It seems that the auxin homeostasis regulation system during embryogenesis is rather robust, since mutations in *AtPIN1* or *AtPIN4* genes results only in less pronounced and not fully penetrating defects in embryo development (Okada *et al.*, 1991; Friml *et al.*, 2002b).

Outlook

It seems that PAT is now a process very well characterized from a physiological point of view. The use of genetic approaches resulted in an extensive collection of mutants defective in PAT. The main recent breakthrough in the field happened when some of these mutants were characterized at the molecular level, and *AUX1* and *PIN* gene families encoding putative auxin influx and efflux carrier components have been identified. The main task for the near future is the biochemical establishment of auxin transport function and transport properties of these proteins and the identification of their interaction partners. Also the isolation and characterization of remaining members of *AUX* and *PIN* families in *Arabidopsis* is awaited in order to describe the whole network of these carriers in plants, their possible functional redundance and their expression and activity regulation in a developmental context. However, one of the most interesting areas of PAT research lies probably in their cellular analysis. Here the polarly localized PIN and AUX proteins provide ideal tools to study cellular polarity establishment, a process poorly understood in higher plants. For PAT research, however, we are awaiting with curiosity further studies on the relationship between the vesicle trafficking pathway and auxin efflux.

Acknowledgements

We are very grateful to Eva Benková, Malcolm Bennett, Niko Geldner, Matthias Godde, Thorsten Hamann and Gerd Jürgens for helpful discussions and critical reading of the manuscript. We

also acknowledge support by DAAD (J.F.) and the Schwerpunktprogramme 'Phytohormone' by the Deutsche Forschungsgemeinschaft.

References

Baker, D.A. 2000. Long-distance vascular transport of endogenous hormones in plants and their role in source: sink regulation. Israel J. Plant Sci. 48: 199–203.

Benjamins, R., Quint, A., Weijers, D., Hooykaas, P. and Offringa, R. 2001. The PINOID protein kinase regulates organ development in *Arabidopsis* by enhancing polar auxin transport. Development, 28: 4057–4067.

Bennett, M.J., Marchant, A., Green, H.G., May, S.T., Ward, S.P., Millner, P.A., Walker, A.R., Schulz, B. and Feldmann, K.A. 1996. *Arabidopsis AUX1* gene: a permease-like regulator of root gravitropism. Science 273: 948–950.

Benning, C. 1986. Evidence supporting a model of voltage-dependent uptake of auxin into cucurbita-pepo vesicles. Planta 169: 228–237.

Berleth, T. and Jürgens, G. 1993. The role of the MONOPTEROS gene in organizing the basal body region of the *Arabidopsis* embryo. Development 118: 575–587.

Bernasconi, P., Bhavesh, P.C., Reagan, J.D. and Subramanian, M.V. 1996. The N-naphthylphthalamic acid-binding protein is an integral membrane protein. Plant Physiol. 111: 427–432.

Cambridge, A.P. and Morris, D.A. 1996. Transfer of exogenous auxin from the phloem to the polar auxin transport pathway in pea (*Pisum sativum* L.). Planta 199: 583–588.

Camus, G. 1949. Recherches sur le role de bourgeons dans les phénomènes de morphogènes. Rev. Cytol. Biol. Vég. 11: 1–195.

Casimiro, I., Marchant, A., Bhalerao, R.P., Beeckman, T., Dhooge, S., Swarup, R., Graham, N., Inzé, D., Sandberg, G., Casero, P.J. and Bennett, M. 2001. Auxin transport promotes *Arabidopsis* lateral root initiation. Plant Cell.13: 843–852.

Chen, R., Hilson, P., Sedbrook, J., Rosen, E., Caspar, T. and Masson, P.H. 1998. The *Arabidopsis thaliana AGRAVITROPIC1* gene encodes a component of polar auxin-transport efflux carrier. Proc. Natl. Acad. Sci. USA 95: 15112–15117.

Christensen, S.K., Dagenais, N., Chory, J. and Weigel, D. 2000. Regulation of auxin response by the protein kinase PINOID. Cell 100: 469–478.

Cox, D.N. and Muday, G.K. 1994. NPA binding-activity is peripheral to the plasma membrane and is associated with the cytoskeleton. Plant Cell 6: 1941–1953.

Darwin, C. and Darwin, F. 1881. The power of movement in plants (Deutsche Übersetzung: Das Bewegungsvermögen der Planze). Darwins gesammelte Werke, Bd. 13, Schweizer-bart'sche Verlagsbuchhandlung, Stuttgart, Germany.

Davies, P.J. 1995. Plant Hormones: Physiology, Biochemistry and Molecular Biology. Martinus Nijhoff, Dordrecht, Netherlands.

Delbarre, A., Muller, P. and 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.

Epel, B.L., Warmbrodt, R.P. and Bandurski., R.S. 1992. The ethylene signal transduction pathway in plants. Science 268: 667–675.

Firn, R.D., Wagstaff, C. and Digby, J. 2000. The use of mutants to probe models of gravitropism. J. Exp. Bot. 51: 1323–1340.

Friml, J., Wisniewska, J., Benková, E., Mendger, K. and Palme, K. 2002a. Lateral relocation of auxin efflux regulator AtPIN3 mediates tropism in *Arabidopsis*. Nature: in press.

Friml, J., Benková, E., Blilou, I., Wisniewska, J., Hamann, T., Ljung, K., Woody, S., Sandberg, G., Scheres, B., Jürgens, G. and Palme, K. 2002b. AtPIN4 mediates sink driven auxin gradients and patterning in *Arabidopsis* roots. Submitted.

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

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

Gaxiola, R.A., Juan, D.S., Klausner, R.D. and Fink, G.R. 1998. The yeast CLC chloride channel functions in cation homeostasis. Proc. Natl. Acad. Sci USA 95: 4046–4050.

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

Gil, P., Dewey, E., Friml, J., Zhao, Y., Snowden, K.C., Putterill, J., Palme, K., Estelle, M. and Chory, J. 2001. BIG: a calossin-like protein required for polar auxin transport in *Arabidopsis*. Genes Dev. 15: 1985–1997.

Goldsmith, M.H.M. 1977. The polar transport of auxin. Annu. Rev. Plant. Physiol. 28: 439– 478.

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

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

Hertel, R. 1983. The mechanism of auxin transport as a model for auxin action. Z. Pflanzenphysiol. 112: 53–67.

Hobbie, L.J. 1998. Auxin: molecular genetic approaches in *Arabidopsis*. Plant Physiol. Biochem. 36: 91–102.

Imhoff, V., Muller, P., Guern, J. and Delbarre, A. 2000. Inhibitors of the carrier-mediated influx of auxin in suspension-cultured tobacco cells. Planta 210: 580–588.

Katekar, G.F. and Geisler, A.E. 1977. Auxin transport inhibitors. Plant Physiol. 60: 826–829.

Kerk, N. and Feldman, L. 1994. The quiescent center in roots of maize: initiation, maintenance and role in organization of the root apical meristem. Protoplasma 183: 100–106.

Kerk, N.M., Jiang, K. and Feldman L.J. 2000. Auxin metabolism in the root apical meristem. Plant Physiol. 122: 925–932.

Knutson V.P. 1991. Cellular trafficking and processing of the insulin receptor. FASEB J. 5: 2130–2138.

Koizumi, K., Sugiyama, M. and Fukuda, H. 2000. A series of novel mutants of *Arabidopsis thaliana* that are defective in the formation of continuous vascular network: calling the auxin signal flow canalization hypothesis into question. Development 127: 3197–3204.

Lalonde, S., Boles, E., Hellmann, H., Barker, L., Patrick, J.W., Frommer, W.B. and Ward, J. 1999. The dual function of sugar carriers: transport and sugar sensing. Plant Cell 11: 707– 726.

Lehman, A. Black, R. and Ecker, J.R. 1996. *HOOKLESS1*, an ethylene response gene, is required for differential cell elongation in the *Arabidopsis* hypocotyl. Cell 85: 183– 194.

Li, Y., Hagen, G. and Guilfoyle, T.J. 1991. An auxin-responsive promoter is differentially induced by auxin gradients during tropisms. Plant Cell 3: 1167–1176.

Lincoln, C., Britton, J.H. and Estelle, M. 1990. Growth and development of the *axr1* mutants of *Arabidopsis*. Plant Cell 2: 1071–1080.

Ljung, K., Ostin, A., Lioussanne, L. and Sandberg, G. 2001. Developmental regulation of indole-3-acetic acid turnover in Scots pine seedlings. Plant Physiol. 125: 464–475.

Lomax, T.L., Mehlhorn, R.J. and Briggs, W.R. 1985. Active auxin uptake by zucchini membrane vesicles: quantitation using ESR volume and ΔpH determinations. Proc. Natl. Acad. Sci USA 82 1986: 6541–6545.

Lomax, T.L., Muday, G.K. and Rubery, P.H. 1995. Auxin transport. In: P.J. Davies (Ed.) Plant Hormones: Physiology, Biochemistry and Molecular Biology, Kluwer, Dordrecht, Netherlands, pp. 509–530.

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

Maher, E.P. and Martindale, S.J. 1980. Mutants of *Arabidopsis thaliana* with altered responses to auxins and gravity. Biochem. Genet. 18: 1041–1053.

Marchant, A., Kargul, J., May, S.T., Muller, P., Delbarre, A., Perrot-Rechenmann, C. and Bennett, M.J. 1999. AUX1 regulates root gravitropism in *Arabidopsis* by facilitating uptake within root apical tissue. EMBO J. 18: 2066–2073.

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

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

Morris, D. A. and Robinson, J. 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, D.A. and Thomas, 1978. A microautoradiographic study of auxin transport in the stem of intact pea seedlings (*Pisum sativum* L.). J. Exp. Bot. 29: 147–157.

Morris, D.A., Rubery, P.H., Jarman, J. and 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.

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

Newcomb, W. and Wetherell, D.F. 1970. The effects of 2,4,6-trichlorophenoxyacetic acid on embryogenesis in wild type carrot tissue cultures. Bot. Gaz. 131: 242–245.

Normanly, J., Cohen, J.D. and Fink, G.R. 1991. *Arabidopsis thaliana* auxotrophs reveal a tryptophan-independent biosynthetic pathway for indole-3-acetic acid. Proc. Natl. Acad. Sci. USA 90: 10355–10359.

Nowacki, J. and Bandurski, R.S. 1980. Myo-inositol esters of indole-3-acetic acid as seed auxin precursors of *Zea mays* L. Plant Physiol. 65: 422–427.

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

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

Parry, G., Delbarre, A., Marchant, A., Swarup, R., Napier, R., Perrot-Rechenmann, C. and Bennett, M.J. 2001. Novel auxin transport inhibitors phenocopy the auxin influx carrier mutation *aux1*. Plant J. 25: 399–406.

Przemeck, G.K., Mattsson, J., Hardtke, C.S., Sung, Z.R. and Berleth T. 1996. Studies on the role of the *Arabidopsis* gene *MONOPTEROS* in vascular development and plant cell axialization. Planta 200: 229–237.

Raven, J.A. 1975. Transport of indolacetic acid in plant cells in relation to pH and electrical potential gradients, and its significance for polar IAA transport. New Phytol. 74: 163–172.

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

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

Reinhardt, D., Mandel, T. and Kuhlemeier, C. 2000. Auxin regulates the initiation and radial position of plant lateral organs. Plant Cell 12: 507–518.

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

Rubery, P.H. und Sheldrake, A.R. 1974. Carrier-mediated auxin transport. Planta 118: 101– 121.

Ruegger, M., Dewey, E., Hobbie, L., Brown, D., Bernasconi, P., Turner, J., Muday, G.K. and 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.

Sabatini, S., Beis, D., Wolkenfelt, H., Murfett, J., Guilfoyle ,T., Malamy, J., Benfey, P., Leyser, O., Bechtold, N., Weisbeek, P. and Scheres, B. 1999. An auxin-dependent distal organizer of pattern and polarity in the *Arabidopsis* root. Cell 99: 463–472.

Sachs, T. 1989. The development of vascular networks during leaf development. Curr. Top. Plant Biochem. Physiol. 18: 168–183.

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

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

Swarup, R., Friml, J., Marchant, A., Ljung, K., Sandberg, G., Palme, K. and Bennett, M. 2001. Localisation of the auxin permease AUX1 suggests two functionally distinct hormone transport pathways operate in the *Arabidopsis* root apex. Genes Dev., in press.

Uggla, C., Mellerowicz, E.J. and Sundberg, B. 1998. Indole-3-acetic acid controls cambial growth in scots pine by positional signalling. Plant Physiol. 117: 113–121.

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

Went, F.W. 1974. Reflections and speculations. Annu. Rev. Plant Physiol. 25: 1–26.

Wetmore, R.H. and Rier, J.P. 1963. Experimental induction of vascular tissues in callus of angiosperms. Am. J. Bot. 50: 418–430.

Yamamoto, M. and Yamamoto, K.T. 1999. Effects of natural and synthetic auxins on the gravitropic growth habit of roots in two auxin-resistant mutants of *Arabidopsis*, *axr1* and *axr4*: evidence for defects in the auxin influx mechanism of *axr4*. J. Plant Res. 112: 391–396.