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Quick on the Uptake: Characterization of a Family of Plant Auxin Influx Carriers

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Abstract

Auxins are unique among plant signalling molecules in that they are subject to polar transport. Plants employ specialized influx and efflux carrier proteins to transport the auxin indole-3-acetic acid (IAA) in and out of cells. Until recently, auxin transport research has largely focused on the role of the efflux carrier. Given our rapidly advancing knowledge about the development importance of auxin uptake, this review aims to redress the balance by exclusively focusing on the auxin influx carrier. We will review the discovery, molecular characterization, evolution and developmental function(s) of the auxin influx carrier.

Key words: Auxin; Auxin uptake carrier; *Arabidopsis*; AUX1

Background

Plants sense and respond to endogenous signals and environmental cues to ensure optimal growth and development. Plant cells must integrate the myriad transduction events into a comprehensive network of signalling pathways and responses. The phytohormone auxin occupies a central place within this transduction network, frequently acting in conjunction with other signals, to coordinately regulate cellular processes such as division, elongation, and differentiation (reviewed by Swarup and others 2001). As a true hormone, auxin also acts as a noncell autonomous signal. Auxin is synthesized in source organs distinct from those tissues in which the hormone promotes growth and development. For example, shoot-derived auxin has been demonstrated to regulate lateral root emergence (Reed and others 1998; Casimiro and others 2001; Bhalerao and others 2001).

Classical experiments have revealed that auxin is transported in a polar fashion (reviewed by Goldsmith 1977; Kaldeway 1984). In aerial tissues of a wide variety of plant tissues including pea, maize, and *Arabidopsis,* polar auxin transport (PAT) occurs in a basipetal (apex to base) direction (Johnson and Morris 1987; Parker and Briggs 1990; Parry and others 2001). In contrast, two distinct streams of PAT have been described in maize and *Arabidopsis* root tissues (Scott and Wilkins 1968; Rashotte and others 2000). Researchers have observed that auxin moves

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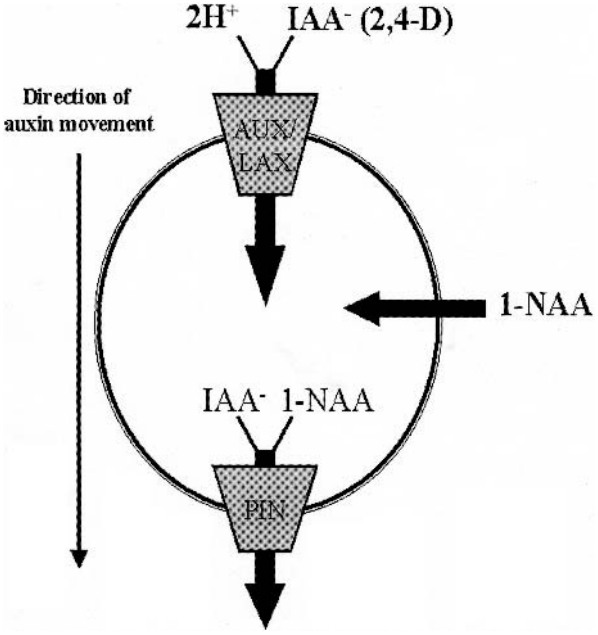

Figure 1. Cellular mechanism of auxin transport. The auxins 2,4-D and IAA can enter cells via an auxin influx carrier whereas 1-NAA can pass directly into the cell (Delbarre and others 1996). Both 1-NAA and IAA are substrates for auxin efflux carrier proteins (Palme and Galweiler 1999).

in both an acropetal (base to tip) and basipetal direction (Scott and Wilkins 1968; Davies and others 1972, 1976; Tsurumi and others 1978; Rashotte and others 2000). In addition, there is growing evidence in *Arabidopsis* for a non-PAT mode of auxin redistribution from auxin source tissues such as young leaves (Ljung and others 2001; Marchant and others, in press) to the root via the phloem (Swarup and others 2001). Intuitively, the speed and distance often required to move auxin from shoot meristem to root tip, particularly in larger plants, could only realistically be achieved via the vascular system (Baker 2000). Nevertheless, the majority of auxin transport research to date has focused on polar auxin transport because inhibitors (Katekar and Geissler 1980) and *Arabidopsis* mutants (Palme and Galweiler 1999) have been identified that disrupt this process, causing pleiotropic developmental defects such as aberrant lateral shoot and agravitropic root phenotypes.

At the cellular level, auxin is transported through a combination of diffusion and carrier-mediated mechanisms (Delbarre and others 1996; Figure 1). As a weak acid (pKa = 4.6), indole-3-acetic acid (IAA) is rapidly protonated in the apoplastic space, then taken up by the plant cell either by diffusion or via an auxin uptake (influx) carrier. Once within the plant cytosol (pH 7) the dissociated IAA^- is removed by a distinct auxin efflux carrier (Delbarre and others 1996). The basipetal polarity of auxin transport in shoot tissues has been proposed to arise from the asymmetric plasma membrane (PM) localization of the auxin efflux carrier (Rubery and Sheldrake 1974; Raven 1975; Figure 1). Consistent with the predictions made in the chemiosmotic hypothesis, immunolocalization studies have revealed a basal localization for the putative auxin efflux carrier protein *At*PIN1 in the PM of specialized xylem parenchyma cells that mediate polar auxin transport (Galweiler and others 1998). The chemiosmotic hypothesis also predicted that the auxin influx carrier facilitates PAT. However, the role of the auxin influx carrier during PAT is questionable since inhibitors designed to block auxin uptake do not to disrupt the polar movement of radiolabelled auxin in *Arabidopsis* influorescence tissues (Parry and others 2001). Instead, the auxin influx carrier appears to regulate processes that require localized auxin redistribution such as *Arabidopsis* root gravitropism (Parry and others 2001).

Until recently, auxin transport research has largely focused on the role of the efflux carrier. Given our rapidly advancing knowledge about the auxin influx carrier, this review aims to redress the balance by exclusively focusing on the auxin influx carrier. We will review the discovery, molecular characterization, evolution, and developmental function(s) of the auxin influx carrier. Readers interested in further details about the auxin efflux carrier are recommended to read the recent review by Galweiler and Palme 1999.

Discovery of the Auxin Influx Carrier

Experimental evidence demonstrating the existence of an auxin influx carrier was first reported by Rubery and Sheldrake (1974) who described a saturable component for auxin uptake in suspension cells. Nevertheless, the auxin influx carrier has been difficult to study because its natural substrate, IAA, readily diffuses in and out of plant cells and is a substrate for both auxin carriers (Sabater and Rubery 1987). Delbarre and others (1996) used tobacco cell cultures to show that the uptake of 2,4-dichlorophenoxyacetic acid (2,4-D) is saturable whereas 1-naphthaleneacetic acid (1-NAA) uptake is not. This difference reflects that the lipophilic 1-NAA is able to passively enter cells whereas 2,4-D requires the activity of an active carrier protein (Delbarre and others 1996). The authors exploited the observed substrate specificity of the auxin influx car-

rier to estimate that carrier-mediated uptake accounted for over three-quarters of auxin accumulated by tobacco suspension-cultured cells (Delbarre and others 1996).

Lomax and co-workers employed sealed zucchini hypocotyl membrane vesicles to study the mechanism of ^{14}C-IAA uptake (Hertel and others 1983; Lomax and others 1985; Lomax 1986). IAA uptake was observed to be saturable and selective for biologically active auxins in this *in vitro* experimental system (Hertel and others 1983; Lomax 1986). When used in conjunction with electron spin resonance (ESR) probes that monitor ΔpH, IAA uptake was reduced by nigericin, an ionophore which totally dissipated the pH gradient (Lomax and others 1985). However, the protonophore FCCP was also capable of reducing IAA accumulation to background levels, despite only lowering the pH gradient to where the proton motive force was reduced to zero by the membrane electrical potential (Lomax and others 1985). Hence, IAA uptake appeared to be dependent on the proton motive force rather than ΔpH. The authors also obtained evidence that the auxin uptake carrier acted as a proton symporter, mobilizing $2H^+/IAA^-$, a conclusion later confirmed by Sabater and Rubery (1987).

Biochemical and physiological studies of auxin transport have mainly addressed the mechanisms underlying auxin efflux, as most known auxin transport inhibitors target the phytotropin-binding site in the auxin efflux complex (Rubery 1990. Equivalent pharmacological studies of carrier-mediated auxin uptake have been hampered by the absence of suitable auxin influx carrier inhibitors. Several workers have employed 2-naphthalene acetic acid (2-NAA) to inhibit auxin influx carrier activity in tissue fragments (Sussman and Goldsmith 1981) and isolated membrane vesicles (Benning 1986; Jacobs and Hertel 1978). However, 2-NAA also perturbs auxin efflux (Delbarre and others 1996) and exhibits weak auxin activity (Katekar 1979). Imhoff and others (2000) have screened a large number of aryl and aryloxyalkylcarboxylic acids for selective reduction of carrier-mediated 2, 4-D uptake in suspension-cultured tobacco cells. 1-naphthoxyacetic acid (1-NOA) and 3-chloro-4-hydroxyphenylacetic acid (CHPAA) were selected, based on their capacity to inhibit auxin influx carrier activity at micromolar concentrations. Parry and others (2001) recently evaluated the effects of both compounds on wild type *Arabidopsis* seedlings, demonstrating that CHPAA and 1-NOA treatments phenocopied the *Arabidopsis auxl* mutant. Similarly, the naturally occurring compound chromasoponin (CSI) causes an agravitropic phenotype in wild type *Arabidopsis* seedlings (Rahman and others 2001). Intriguingly, the authors reported that CSI treatment could rescue the agravitropic phenotype of the *aux1-7* mis-sense allele, but not the *aux1-22* deletion allele, suggesting that CSI directly interacts with the AUX1-7 protein to restore function.

Molecular Characterization of the Auxin Influx Carrier

Research on the auxin influx carrier at the molecular level has largely employed a genetic approach in the model plant *Arabidopsis thaliana.* Maher and Martindale (1980) originally identified the *aux1* mutation in a screen for mutant seedlings whose root elongation was less sensitive to the auxin 2,4-D. Evans and others (1994) observed that *aux1* roots exhibited a delay in their response to inhibitory levels of exogenous auxin, compared with wild type and the auxin signalling mutants, *axr1* and *axr2.* The authors proposed that the delay was most consistent with *aux1* being defective in IAA uptake. Bennett and colleagues (1996) subsequently isolated the *AUX1* gene and reported that it encoded a putative auxin permease.

To date, multiple lines of evidence have been reported by ourselves and others which support the fact that the AUX1 protein acts as a component and/or regulator of the auxin influx carrier. Elongation growth bioassays have revealed that *aux1* roots are selectively resistant to IAA and 2,4-D but not 1-NAA (Marchant and others 1999; Yamamoto and Yamamoto 1998), mirroring the known substrate specificity of the auxin influx carrier (Delbarre and others 1996). The differential auxin-resistant phenotype of *aux 1* roots can be phenocopied in wild type *Arabidopsis* seedlings using the auxin influx carrier inhibitors, 1-naphthoxyacetic acid (1-NOA) and CHPAA (Parry and others 2001).

Mutations in the *AUX1* gene cause an agravitropic phenotype (Maher and Martindale 1980) that can be phenocopied in wild type seedlings using the auxin influx carrier inhibitors 1-NOA and CHPAA (Parry and others 2001). Similarly, CSI causes an agravitropic phenotype that is likely to result from a direct interaction with the AUX1 protein (Rahman and others 2001). Swarup and others 2001 have reported that the *aux1* agravitropic root phenotype is caused by the disruption of basipetal auxin transport in the mutant, as monitored by the loss of expression of the auxin responsive *IAA2::uidA* reporter in lateral root cap (LRC) and distal elongation zone (DEZ) tissues. Rashotte and others (2000) have confirmed this conclusion, detecting a reduction in the

basipetal movement of radiolabelled IAA in *aux1* root apical tissues.

The *Arabidopsis* sterol biosynthesis mutation *hydra2* has been observed to suppress the *aux1* agravitropic mutant phenotype (Martin Souter and Keith Lindsay, University of Durham UK, pers. comm.). This suppressor effect is likely to result from changes in the composition of the *hydra2* plasma membrane that enhances root cell permeability to endogenous IAA in the *aux1 hydra2* double mutant, thereby bypassing the *aux1* defect in auxin uptake (Marchant and others 1999). Likewise, the *aux1* and 1-NOA-induced agravitropic phenotypes can be rescued using the membrane permeable auxin 1-NAA (Yamamoto and Yamamoto 1998; Marchant and others 1999; Parry and others 2001). In contrast, auxins that require influx carrier activity such as 2,4-D are unable to rescue *aux1* root gravitropism (Yamamoto and Yamamoto 1998; Marchant and others 1999; Parry and others 2001). Using the *IAA2::uidA* reporter, Swarup and others 2001 demonstrated that 1-NAA-mediated rescue of *aux1* root gravitropism resulted from the restoration of basipetal auxin transport (whereas 2,4-D was unable to).

Bennett and others (1996) reported that the AUX1 gene had 21% identity and 48% similarity to members of a family of plant and fungal amino acid permeases (AAP). The conservation of homology extends across almost the entire open reading frames, suggesting that the AUX1 and AAP sequences share a common domain structure and hence transport function. Indeed, hydropathy plots predict that the AUX1 protein contains 11 transmembrane spanning domains (Bennett and others 1996) as proposed for the AAP family (Younger and others 1999) and directly demonstrated from studies on AAP1 (NAT2) protein topology (Chang and Bush 1997). The observed homology between the AAP family and the putative auxin permease AUX1 is consistent with transport studies that have demonstrated that the auxin influx carrier and AAP proteins function as proton-symporters (Sabater and Rubery 1987; Ortiz-Lopez and others 2000). The conservation of structure between AUX1 and AAPs is not surprising given the common indole side chain of their proposed substrates, IAA and tryptophan, respectively (Bennett and others 1996). Nevertheless, transport assays on *aux1* roots did not observe a reduction in tryptophan uptake. Instead, mutant roots accumulated 50 % less 2,4-D than wild type (Marchant and others 1999), suggesting that AUX1 has specificity for auxin alone.

Western immuno-detection experiments have observed that both native and recombinant AUX1 migrate at an estimated molecular weight of 42 kD, considerably faster than the predicted value of 54 kD, as has been observed for other permease proteins (Stoltz and others 1994). The estimated molecular weight of AUX1 is highly significant since Hicks and others (1989) reported photo-affinity labelling two low abundance 40 and 42 kD plasma membrane polypeptides using azido-IAA. Azido-IAA labelling was competitively inhibited by selected auxins that shared specificity for the auxin influx (but not efflux) carrier (Hicks and others 1989). Later studies demonstrated that the labelled polypeptides were integral membrane proteins that form a multimeric complex of approximately 300 kD (Hicks and others 1993). AUX1 has also been demonstrated to be localized in the membrane during subcellular fractionation experiments on selected *Arabidopsis* tissues (J.Kargul, A. Yemm, R.Napier, M.J.Bennett, unpub. results), immunolocalization studies in *Arabidopsis* roots (Swarup and others 2001) and recombinant expression of AUX1 in insect cell lines (J. Kargul, A. Yemm, R.Napier, M.J.Bennett, unpub. results). Interestingly, intragenic complementation experiments using an *aux1* allelic series of missense mutations suggest that the AUX1 functions as a multimeric protein (R. Swarup, A. Marchant and M. J. Bennett, unpub results).

Though this list represents a significant body of indirect evidence in favor of AUX1 encoding an auxin uptake carrier, we must await direct biochemical evidence to provide final confirmation of our model.

Evolution of a Family of Plant Auxin Influx Carriers

AUX1 has recently been included in the ATF (amino acid transporter) family of proteins (Young and others 1999; Ortiz-Lopez and others 2000; Figure 2). The ATF family contains five distinct subclasses, AUX1, the amino acid permeases (AAP), the lysine histidine transporters (LHTs), the proline transporters (ProT) and the new aromatic and neutral amino acid transporter class (ANT). The ANT1 protein has been identified as the first permease to transport both aromatic and neutral amino acids (Chen and others 2001). In addition, the authors report that expression of ANT1 in yeast cells facilitates the uptake of both 2, 4-D and IAA, indicative of a potential role as an auxin carrier. However, further biochemical and genetic data is required before the ANT family of proteins is demonstrated to have a physiologically significant auxin transport function *in planta.*

Young and others (1999) speculated that many

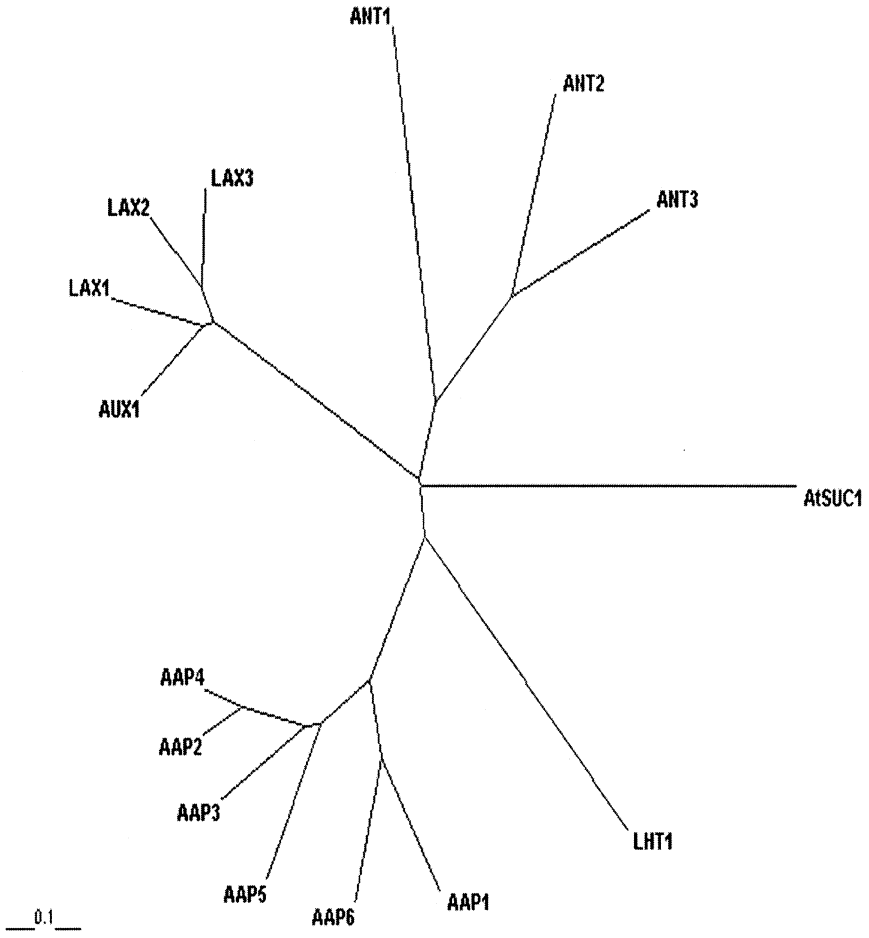

Figure 2. Phylogenetic tree comparing AUX1 with other *Arabidopsis* permeases. Phylogenetic analysis was carried out using the Clustal X alignment tool (Higgins and Sharp 1989) on the protein sequences of AUX1 and other permease proteins. ANT2 and ANT3 were obtained following a BLAST search using the amino acid sequence of the ANT1 protein (Chen and others 2001). Accession numbers: AUX1, X98772; LAX1, AJ249442; LAX2, AJ243221; LAX3, AC012193; ANT1, U39783; ANT3, AL035539; AAP1, L16240; AAP2, X71787; AAP3, X77499; AAP4, X77500; AAP5, X77501; AAP6, X95736; LHT1, U39782 and AtSUC1, X75365. Bac Locus of ANT2, K2A18_5.

eukaryotic and prokaryotic amino acid permeases share a common evolutionary ancestry. Interestingly, phylogenetic analyses have indicated that AUX1 is more closely related to two proteins of unknown function from the nematode, *C.elegans,* than the AAP permeases of *Arabidopsis* (Young and others 1999). It is therefore most likely that the AUX family of proteins arose from a common permease ancestor than from a change of function of an already defined AAP-like protein and may reflect the early evolution of auxin-regulated development in plants. Interestingly, we have recently identified a *Physcomitrella* sequence that is highly similar to AUX1 (T.Allen, R. Swarup, and M.J. Bennett, unpub results), indicating that this ATF subclass already existed when nonvascular plants evolved.

We and others have identified AUX1 related sequences in a wide range of angiosperms including *Brassica* (T.Allen, M.J.Bennett, G.King, unpub results), poplar (J. Schrader, R.Bhalerao, M.J.Bennett, G. Sandberg, unpub results), cherry (N. Grant, N. Hammat, S. May, M.J.Bennett, unpub results), wild strawberry (T. Martucci, M.J.Bennett, K. Manning, unpub results), rice (S. May, M.J.Bennett, unpub results) and maize (Hochholdinger and others 2000). The *AUX1* sequence is most closely related to three other sequences in the *Arabidopsis* genome that are termed *LAX1, LAX2* and *LAX3* (Like *AUX*1). Though the *Arabidopsis AUX/LAX* genes exhibit a high degree of amino acid sequence conservation (Figure 3), the number and position of each gene's introns may hint at the evolutionary relationship between the four family members (Figure 4). The first five exons at the N-terminus of the *AUX/LAX* genes are almost identical; however the exon/intron structure diverge at the C-terminal end of the genes. In *AUX1* and *LAX1* genes the C-terminal coding sequences are split by two introns, the *LAX3* gene contains only intron, and-*LAX2* has no intron at this position (Figure 4). Assuming that the addition of introns is a measure of evolving genome complexity, *LAX2* would represent the founding member of the *AUX/LAX* family, whereas *AUX1, LAX1,* and *LAX3* sequences would have evolved at later stages through gene/genome duplication. In *Brassica oleaceae,* we have recently observed a threefold increase

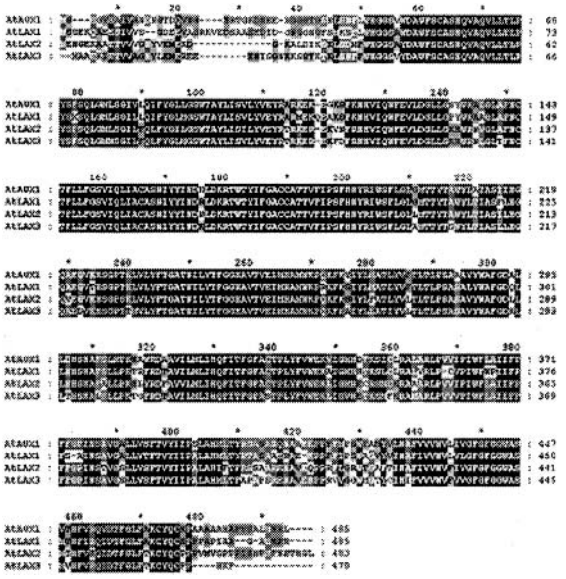

Figure 3. Amino acid sequences are highly conserved within the AUX/LAX family. Amino acid alignment of the AUX/LAX family of proteins. Alignment was carried out using the Clustal X alignment tool (Higgins and Sharp 1989). Black and grey shading correspond to regions of identity among the four family members. For accession numbers see Figure 2.

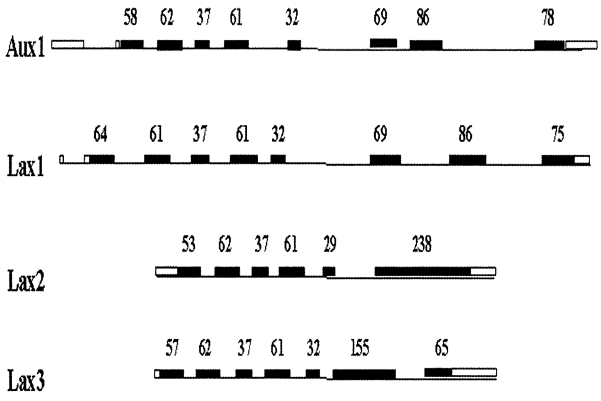

Figure 4. Arrangment of introns within the AUX/LAX family. Sequence runs left to right from N to C terminus. Coding and noncoding exons are represented by black and white boxes, respectively. Introns are represented by the black line. Figures indicate the number of amino acid residues coded by each exon.

in *AUX/LAX* gene copy number (T.Allen, M.J.Bennett, G.King, unpubl results), reflecting the triplication of the *Brassica* genome. However, parallel studies in other diploid dicotyledenous plant species such as wild strawberry (T. Martucci, M.J. Bennett, K.Manning, unpub results) and poplar (*Populus tremula;* J. Schrader, R. Bhalerao, M.J. Bennett, G. Sandberg, unpub results) have revealed that they share a similar copy number to *Arabidopsis,* suggesting that gene duplication occurred prior to angiosperm evolution.

AUX1 shares between 73% and 82% identity at the amino acid level with other *LAX* sequences (Figure 3). Such levels of conservation suggest that there is a strong selective pressure to maintain the primary sequence. Indeed, characterization of an *aux1* allelic series (Marchant and Bennett 1998; R. Swarup, A. Marchant, M.J. Bennett, unpub results) has observed that even subtle amino acid substitutions can cause significant effects on the penetrance of the mutant phenotype, presumably due to changes in AUX1 protein activity. More generally, the strong conservation of amino acid similarity between *AUX/LAX* family members (Figure 3) is indicative of retention of a common biochemical function. Indeed, knockout mutations in selected *LAX* genes have been observed to cause alterations in auxin-regulated developmental traits (K.Swarup, R. Swarup, N.James, N.Graham, M.J.Bennett, unpub results). The apparent lack of functional redundancy between *AUX1* and other *LAX* family members can be explained by the non-overlapping patterns of expression observed in *AUX/LAX* promoter-driven GUS transgenes (N. James M.J.Bennett, unpub results).

Developmental Functions of the Plant Auxin Influx Carrier

Until relatively recently, the developmental function(s) of the auxin influx carrier was unclear (Lomax and others 1995). However, detailed characterization of the *aux1* mutant phenotype has revealed diverse roles for the putative auxin influx carrier during a wide selection of developmental processes in *Arabidopsis.* These include root gravitropism (Maher and Martindale 1980); root thigmotropism (Okada and Shimura, 1990); auxin and ethylene sensitive root elongation growth (Picket and others 1990); root hair differentiation (Masucci and Schiefelbein 1994; Grebe and Scheres pers. comm.); lateral root development (Hobbie and Estelle 1995; Marchant and others in press) and leaf expansion (Timpte and others 1995).

One developmental scenario would involve the expression of an auxin influx carrier in one cell versus another, since it would provide the former cell with the ability to accumulate higher levels of auxin. Our studies have revealed that *Arabidopsis* lateral root development represents such an example (Marchant and others in press). We have used *an A*

UX1::uidA reporter construct to observe that expression of the putative auxin influx carrier is induced during a late phase of stage I lateral root primordia development. Interestingly, loss of AUX1 expression in the *aux1* mutant leads to a delay in the stage I to stage II transition of lateral root primordia (Marchant and others in press). We conclude that this, as with other early stages of *Arabidopsis* lateral root development, represents an auxin-dependent process (Casimiro and others 2001).

Expression of the auxin influx carrier in several adjacent cells would enable auxin to be relayed from cell to cell. Root gravitropic signal transduction in *Arabidopsis* provides an example of how the auxin influx carrier can be employed to facilitate such an intercellular auxin relay. AUX1 expressing lateral root cap cells (LRC) have been proposed to facilitate basipetal auxin transport between the gravity-sensing columella cells and the gravitropic-responsive tissues of the distal elongation zone (DEZ) (Marchant and others 1999). Consistent with this model, mutations within AUX1 disrupt basipetal auxin transport (Rashotte and others 2001; Swarup and others 2001). Localization studies using a fully functional epitope-tagged version of AUX1 (termed HA-AUX1) have revealed that the protein is localized symmetrically in LRC cells (Swarup and others *submitted*). We conclude from this observation that AUX1 simply facilitates the movement of auxin between LRC cells but does not specify the basipetal polarity of auxin transport in this tissue. This conclusion agrees with earlier studies that reported the membrane-permeable auxin 1-NAA was able to bypass the *aux1* defect and fully rescue the mutant agravitropic phenotype (Marchant and others 1999; Yamamoto and Yamamoto 1998). Instead, Marchant others (1999) proposed that auxin efflux carrier activity provided the basipetal direction of polar auxin transport in root apical tissues since the phytotropin 1-napthylphthalamic acid (NPA) is able to block the 1-NAA rescue of the *aux1* agravitropic root phenotype. Immunolocalization studies have revealed that the auxin efflux carrier *At*PIN2 is asymmetrically localized in *Arabidopsis* LRC and DEZ epidermal/cortical plasma membranes (Friml and others Palme pers. comm.; Muller and others 1998), thereby providing a likely molecular mechanism for the observed basipetal auxin movement in these tissues (Rashotte and others 2000).

The examples given above highlight that AUX1 facilitates local auxin redistribution between a group of plant cells and/or tissues. However, localization of HA-AUX1 in the *Arabidopsis* root apex has revealed a novel role in a phloem-based long distance auxin transport pathway (Swarup and others 2001). Significant concentrations of free IAA (~1μM) have been detected in the castor bean phloem (Baker 2000). Nevertheless, the cellular pathway for post-phloem transport in roots of any plant species remains to be established (Bret-Harte and Silk 1994; Fisher and others Oparka 1996). Protophloem cell files represent a likely route, but these cells would require carriers to unload IAA from the phloem since they retain an intact PM prior to sieve plate formation (Esau 1965). Localization of HA-AUX1 in the protophloem cell PM in this respect is therefore significant. Interestingly, HA-AUX1 localization in the protophloem cell PM appeared asymmetric. Double-labeling experiments designed to localize HA-AUX1 relative to the asymmetric root stele marker, *At*PIN1 have revealed that the proteins were preferentially targeted to the upper and lower protophloem PM domains, respectively. Localization of AUX1 to the upper PM of protophloem cell files would be expected to facilitate the acropetal, post-phloem movement of IAA. Direct hormone measurements using GC-SRM-MS analysis has demonstrated that IAA accumulation in *aux1* mutant root apices was impaired, consistent with an AUX1 phloem unloading function (Swarup and others 2001).

AUX1 also appears to facilitate hormone loading in young leaf primordia that represent important IAA source organs (Marchant and others in press). GC-SRM-MS measurements have revealed that there are quantitative differences in IAA levels between *aux1* and wild-type leaves. To provide a greater level of spatial resolution, the expression of the auxin inducible *IAA2::uidA* marker (Luschnig and others 1998; Swarup and others 2001) was examined in either a wild-type *Arabidopsis* or *aux1* background. In wild-type primary leaves, expression was localized predominantly to higher order vascular elements, whereas in *aux1* leaves, GUS staining within vascular elements was greatly reduced (despite containing an elevated level of IAA within aerial tissues). The altered pattern of *IAA2::uidA* expression in *aux1* leaves is most consistent with impaired vascular loading rather than a developmental defect given that *aux1* leaves exhibit a wild-type vascular pattern (Marchant and Bennett, unpublished results). A threshold level of IAA is likely to be required to obtain detectable *IAA2::uidA* expression in *Arabidopsis* leaf tissues, based on the behavior of the endogenous *IAA2* gene (Abel and others 1995). Hence, defective auxin loading in *aux1* leaf vascular tissues would fail to elevate IAA levels sufficiently to induce *IAA2::uidA* expression.

To date, polar auxin transport in the xylem parenchyma has been assumed to represent the sole conduit for IAA transport to roots. Our results ques-

tion the traditional view that polar auxin transport represents the long distance IAA transport pathway in plants. We conclude from GC-SRM-MS measurements in *aux1* that a significant proportion of root apical IAA is unloaded by AUX1 from a phloem source (Swarup and others 2001). Parallels can be drawn between the phloem transport functions of AUX1 and other carriers such as the related AAP family. It is tempting to speculate that the AUX1 sequence has evolved to facilitate IAA transport via the phloem. Nevertheless, in addition to its novel phloem-related unloading function, we have demonstrated that AUX1 facilitates auxin transport in columella/lateral root cap/central elongation zone tissues. Therefore, AUX1 performs a dual function, facilitating acropetal and basipetal auxin transport within inner and outer tissues of the root apex, respectively.

In summary, given the rich molecular resources available, *Arabidopsis* will continue to represent the model experimental system to study auxin influx carrier function. Studies on AUX1 and related LAX sequences have and will no doubt continue to provide insight into the developmental processes regulated by proteins in *Arabidopsis.* Likewise, AUX1 localization in *Arabidopsis* root protophloem cell files demands a reevaluation of current thinking about the cellular pathway(s) of auxin transport that operates in plant species. Nevertheless, we must not overlook the rich diversity of auxin influx carrier-based signaling mechanisms that are likely to have evolved in other plant species such as the moss *Physcomitrella* and endeavor to adopt acomparative approach in our future research.

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