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Going the distance with auxin: unravelling the molecular basis of auxin transport

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THE ROYAL SOCIETY

Going the distance with auxin: unravelling the molecular basis of auxin transport

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Auxin represents one of the most important classes of signalling molecules described in plants. Auxins regulate several fundamental cellular processes including division, elongation and differentiation. Indole-3-acetic acid (IAA), the principal form of auxin in higher plants, is first synthesized within young apical tissues, then conveyed to its basal target tissues by a specialized delivery system termed polar auxin transport. The polarity of IAA movement represents one of the most novel aspect of auxin signalling. IAA transport has been demonstrated to involve auxin influx and efflux carrier activities. The adoption of a mutational approach in the model plant *Arabidopsis thaliana* has led to the identification of a number of genes which encode components for, or regulate the activity of, the auxin transport machinery. This paper will review the advances being made in identifying and characterizing these auxin transport-related gene products and discuss their importance within the context of *Arabidopsis* development.

Keywords: *Arabidopsis*; phytohormone; indole-3-acetic acid; auxin transport; embryogenesis; vegetative development

1. INTRODUCTION

The hormone auxin influences many aspects of plant development. For example, auxins regulate several fundamental cellular processes including division, elongation and differentiation (figure 1). Auxin is unique amongst plant hormones in demonstrating a polarity in its movement. Auxin moves in a basipetal direction within excised plant tissue segments (reviewed by Goldsmith (1977)). IAA, the principal form of auxin in higher plants, is first synthesized within young apical tissues, then conveyed to its basal target tissues by a specialized delivery system termed polar auxin transport. Polar auxin transport is distinct from faster vascular-based mechanisms, moving at a velocity of *ca.* 5–10 mm per hour. The relatively slow movement of IAA is proposed to reflect the energy-dependent transport of the hormone between specialized transport cells. IAA moves from cell to cell through the combined activities of the auxin influx and efflux carrier complexes (see figure 2). Rubery, Sheldrake and Raven have proposed within their chemiosmotic hypothesis that the polarity of IAA transport reflects the asymmetric subcellular distribution of auxin efflux (and possibly influx) carrier proteins within specialized transport cells (Rubery & Sheldrake 1974; Raven 1975).

The physiological characterization of polar auxin transport has been greatly facilitated by the identification of a number of synthetic and naturally occurring inhibitors (Katekar & Geissler 1977; Jacobs & Rubery 1988). All transport inhibitors described to date act primarily by blocking auxin efflux (rather than influx) carrier activity (see Lomax *et al.* 1995). Several different classes of auxin transport inhibitors have been described, based on their effects on gravitropism, plant morphology or their own auxin-like properties. These include phytotropins, such as 1-N-naphthylphthalamic acid (NPA); morphactins like 2-chloro-p-hydroxyfluorene-9-carboxylic acid; flavanoids such as quercetin; and 2,3,5,-triiodo-benzoic acid (TIBA). Quercetin and NPA are proposed to block polar auxin movement through their affinity for the so-called phytotropin binding site (Jacobs & Rubery 1988). Bernasconi *et al.* (1996) concluded that the phytotropin binding site in zucchini is an integral membrane protein, which may represent the efflux carrier. However, other workers have observed that the phytotropin binding site is physically distinct from the transmembrane efflux carrier. For example, Muday and co-workers have localized the NPA binding site to the cytoplasmic face of the plasma membrane which is associated with the cytoskeleton (Cox & Muday 1994; Dixon *et al.* 1996). Morris *et al.* (1991) have proposed that the efflux carrier comprises (at least) three polypeptides; a transmembrane carrier protein; an NPA binding protein; and a third, labile component. The authors demonstrated that treatment of zucchini hypocotyl segments with protein synthesis inhibitors blocks the ability of NPA to stimulate the accumulation of tritated IAA without affecting IAA efflux or NPA binding activities. The authors suggest that the protein synthesis inhibitors act by blocking the production of a third, labile component which uncouples the NPA binding and efflux carrier activities.

The ability to gauge the importance of carrier-mediated IAA uptake during polar auxin transport was originally handicapped by the lack of suitable influx carrier-specific inhibitors. The influx carrier was first described by Rubery and co-workers who observed a saturable component for auxin uptake within suspension cell cultures and stem segments (Rubery & Sheldrake 1974; Davies & Sheldrake 1978). Later workers demonstrated the involvement of an

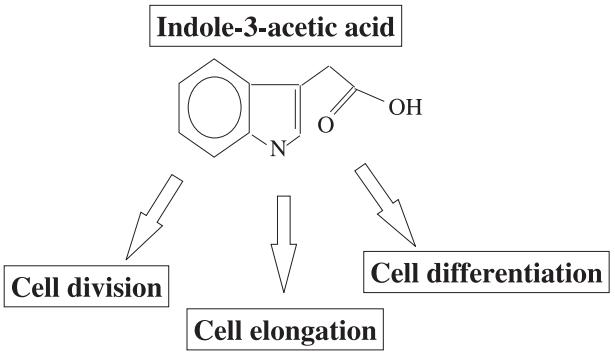

Figure 1. Indole-3-acetic acid (IAA), the major form of auxin in higher plants, regulates several fundamental cellular processes including division, elongation and differentiation.

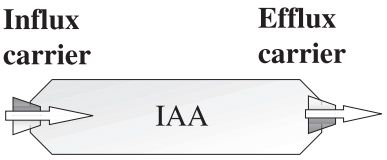

Figure 2. IAA transport is mediated by auxin influx and efflux carriers. The polarity of IAA transport has been proposed to arise from the asymmetric subcellular distribution of auxin efflux (and possibly influx) carrier proteins within specialized transport cells (Rubery & Sheldrake 1974; Raven 1975).

influx carrier in mediating auxin uptake into sealed microsomal vesicles prepared from zucchini hypocotyl tissue (Hertel *et al.* 1983). By using the zucchini model, several groups have demonstrated that carrier-mediated uptake requires a proton motive force because the influx carrier functions electrogenically, transporting one proton with every protonated IAA molecule (Lomax *et al.* 1985; Sabater & Rubery 1987).

2. AUXIN TRANSPORT: ITS IMPORTANCE DURING EMBRYOGENESIS

Auxin transport regulates several important developmental programmes, including embryo patterning. Perturbations in the morphology of somatic and zygotic embryos have been observed when they are incubated *ex planta* with a selection of auxin transport inhibitors. NPA, for example, disrupts the formation of an apical–basal axis at the globular stage of carrot embryogenesis, whereas NPA treatment of wheat embryos induces polyembryo formation with multiple root and shoot meristems. Such observations have led to proposals that IAA-based morphogenic gradients are important during embryo pattern formation. Michalczuk *et al.* (1992) have observed that there is a progressive reduction in the concentration of auxin during later stages of somatic carrot embryogenesis. Such a reduction in hormone concentration may serve as a prerequisite for the formation of carrier-mediated morphogenic gradients of auxin which promote axialization in a developing embryo. Exogenous application of auxins would therefore be expected to disrupt any endogenous gradients in a developing embryo. Fischer & Neuhaus (1996) have done such an experiment and observed that in wheat embryos

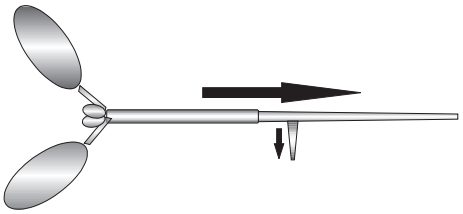

Figure 3. IAA is first synthesized in young apical tissues, then conveyed to its basal target tissues by a specialized delivery system termed polar auxin transport (arrows). The coordinated movement of auxin in the developing seedling regulates post-embryonic organogenic programmes such as lateral root formation.

the attainment of bilateral symmetry can be blocked by exogenous application of either IAA or the synthetic auxins, 2,4-D and 2,3,5-T.

Several *Arabidopsis* mutations which exhibit reduced polar auxin transport have been observed to have altered embryo patterning. The *monopteros* (*mp*) mutant embryo features a disruption in its apical–basal patterning at the globular stage, whereas *pin-formed1* (*pin1*) embryos exhibit a fused cotyledon phenotype (Mayer *et al.* 1991; Liu *et al.* 1993). The altered embryo patterning within these *Arabidopsis* mutants is likely to arise as a result of impaired auxin movement because their aberrant morphologies can be phenocopied when wild-type embryos are treated with auxin transport inhibitors at selected stages of their development. For example, *mp* embryo patterning mimics the disruption of apical–basal patterning at the globular stage of carrot embryogenesis by NPA (Schiavone & Cooke 1987). Likewise, NPA treatment during later stages of *Brassica* embryogenesis can create fused cotyledon structures which phenocopy the *Arabidopsis pin1* mutant (Liu *et al.* 1993). Liu *et al.* (1993) propose that the polar movement of auxin in the apex of the embryo is therefore necessary for the establishment of bilateral symmetry, to facilitate the formation of a pair of cotyledon primordia.

3. AUXIN TRANSPORT: ITS ROLE DURING VEGETATIVE AND REPRODUCTIVE DEVELOPMENT

At the completion of embryogenesis the immature plantlet comprises a rudimentary set of organs including the primary root, hypocotyl and cotyledons. A plant achieves its final adult form by elaborating the seedling's basic morphology using a variety of post-embryonic organogenic programmes. Aerial vegetative and reproductive organs are, for example, post-embryonic products of the shoot apical meristem. Lateral roots likewise enable the developing seedling to elaborate its root architecture. The coordinated movement of auxin within the developing plant regulates many of these post-embryonic organogenic programmes (figure 3).

Lateral roots are derived from pericycle cells which undergo a series of transverse, then periclinal, divisions to give rise to a lateral root primordium (Malamy & Benfey 1997). Auxin transport is clearly necessary for the early stages of lateral root development. For example, blocking polar auxin transport using NPA abolishes lateral root initiation in tomato seedlings (Muday & Haworth 1994).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Similarly, Ruegger *et al*. (1997) have observed that if there are mutations in the *TIR3* gene, both polar auxin transport and lateral root development are significantly reduced. At later stages of their development lateral root primordia appear to acquire the ability to synthesize their own auxin and are thus less reliant on auxin delivered by polar transport (Celenza *et al*. 1995; Laskowski *et al*. 1995).

Auxin transport is also important during reproductive development. For example, floral meristem organization appears to be sensitive to perturbations in auxin transport. Okada *et al*. (1991) observed that wild-type *Arabidopsis* develop severely abnormal, unbranched influorescence structures that usually fail to form floral organs when subcultured in the presence of NPA. When floral organs are able to develop, selected tissues are either missing or structurally aberrant. The *pin1*, *mp* and *pinoid* mutants phenocopy the floral morphological abnormalities induced by NPA (Okada *et al*. 1991; Bennett *et al*. 1995; Przemech *et al*. 1996). Mutations in the *PIN1*, *MP* and *PINOID* genes result in significantly reduced rates of polar auxin transport. Hence, these gene products are likely to influence floral meristem organization by regulating auxin movement either within, or export from, the floral apex.

4. AUXIN TRANSPORT: AN INDUCTIVE SIGNAL DURING TISSUE FORMATION

The coordinated movement of auxin within a developing organ is likely to provide important information for tissue induction. For example, Sachs (1981) has proposed in his canalization model that vascular patterning is an auxin-regulated process involving tracheary elements developing along pathways of canalized auxin movement. Several lines of evidence support this inductive model for auxin-regulated vascular differentiation. First, auxin is capable of promoting vascularization, as demonstrated by the hormone-induced trans-differentiation of *Zinnia elegans* suspension cells to tracheary elements (Burgess & Linstead 1984). Second, tracheary elements have been observed to be induced and align themselves with the direction of artificially imposed auxin gradients (Warren-Wilson *et al*. 1991). Third, mathematical simulations of this inductive model confirm that it provides a valid mechanism for vascular patterning in leaves (Mitchison 1980). The canalization model provides an attractive mechanism to explain the induction of vascular differentiation in developing leaflets. Young leaflets represent the main source of auxin in plants, hence the requirement for auxin export could provide the driving force behind the induction of vascularization during early stages of leaf development.

Several *Arabidopsis* mutants which have a reduced rate of basipetal auxin transport also exhibit alterations in their vascular patterning in leaves. For example, the *lop1* mutant features midvein bifurcation, disorientated axial growth and abnormal leaf cortical cell expansion (Carland & McHale 1996). In contrast, the *mp* mutant has a normal midvein, but develops little or no secondary vascular development in the leaf laminae (Przemeck *et al*. 1996). If the *LOP1* and *MP* gene products encode components for, or regulate the activity of, the auxin transport machinery, the vascular patterning defects may arise as a result of their inability to canalize auxin properly in the developing leaflet.

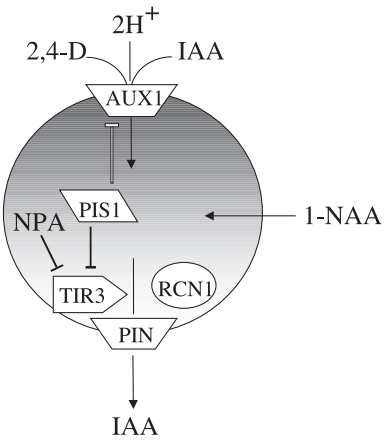

Figure 4. Several putative components of the auxin transport machinery have been identified by using molecular genetic approaches in *Arabidopsis* (see text).

5. DISSECTING THE AUXIN TRANSPORT MACHINERY: CHARACTERIZING ITS MOLECULAR COMPOSITION

Several genes have recently been described in *Arabidopsis* which are likely to encode components of the auxin transport machinery (see figure 4). The *AUX1* gene has been proposed to encode a transmembrane component of the auxin influx carrier (Bennett *et al*. 1996). The influx carrier facilitates the uptake of IAA and synthetic auxins such as 2,4-D, but not the lipophilic auxin, 1-NAA, which enters the cell via diffusion (see Delbarre *et al*. 1996). Such differences in the influx carrier's substrate specificity have proven a useful diagnostic tool to discriminate between mutations which either perturb carrier-mediated uptake or downstream signalling components. Hence, a defect in auxin uptake should selectively impair responses towards IAA and 2,4-D, but not 1-NAA. We have observed, using a root elongation bioassay, that *Arabidopsis aux1* mutants exhibit a reduced response to the auxins, IAA and 2,4-D, yet retain a wild-type sensitivity towards 1-NAA (A. Marchant and M. J. Bennett, unpublished results). Hence, mutations in the *AUX1* gene selectively block the action of auxins which require carrier-mediated uptake. In contrast, other auxin mutations exhibit altered responses to all three auxins, suggesting that they block later steps in the auxin transduction cascade (see Hobbie & Estelle 1994).

The *Arabidopsis AUX1* gene has been isolated by using a gene tagging approach and found to encode a highly hydrophobic polypeptide featuring up to 11 transmembrane-spanning domains. Database searches have identified colinearity between *AUX1* and a family of sequences which encode plant amino-acid permeases (Frommer *et al*. 1993), suggesting that they share a common ancestry and domain structure. Plant amino-acid permeases facilitate the uptake of amino acids into plant cells (Bush 1993). Bennett *et al*. (1996) have suggested that *AUX1* may therefore perform a similar

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

transport role, facilitating the movement of the amino acid-like signalling molecule, IAA. *AUX1* belongs to a family of closely related sequences in *Arabidopsis* (S. T. May, A. Sarjeant, C. Tissier and M. J. Bennett, unpublished data), leading to suggestions that they perform a similar function *in planta*. The several root phenotypes associated with the *aux1* mutation suggest that, at least in these cases, there is no apparent genetic redundancy between family members. Reverse genetic studies are likely to help delineate the precise roles of each family member.

The efflux carrier comprises (at least) three polypeptides; a transmembrane carrier protein; an NPA binding protein; and a third, labile component (Morris *et al.* 1991). The *PIN1 Arabidopsis* gene has been proposed to encode a component of the efflux carrier complex based first on its mutant phenotype, which can be phenocopied by culturing wild-type seedlings in the presence of the efflux inhibitor, NPA, and second, on the significantly reduced rates of polar auxin transport within mutant inflorescence tissues (Okada *et al.* 1991). Galweiler *et al.* (1996) reported the isolation of the *PIN1* gene using a transposon tagging strategy. Significantly, the *PIN1* sequence was found to encode a membrane-localized protein, prompting the suggestion that it may represent the transmembrane component of the auxin efflux carrier (figure 4). Ruegger *et al.* (1997) have recently described the *tir3* mutant, which also exhibits a reduced rate of polar auxin transport. Biochemical studies have observed that the *tir3* mutant contains significantly reduced levels of NPA binding, leading to the suggestion that the *TIR3* gene may encode (or regulate the activity of) the NPA binding protein associated with the efflux carrier (Ruegger *et al.* 1997; see figure 4).

6. DISSECTING THE AUXIN TRANSPORT MACHINERY: UNRAVELLING A NETWORK OF REGULATORS

Several genes which are thought to encode negative regulators of the efflux carrier have recently been described in *Arabidopsis* (Garbers *et al.* 1996; Fujito & Syono 1997; figure 4). The *rcn1* and *pis1* mutations have been selected on the basis of their enhanced sensitivity towards NPA. Both mutants exhibit an enhanced root curling response in the presence of NPA. However, significant differences have been observed for several other phenotypes. For example, *rcn1* root elongation exhibits a wild-type level of response towards NPA, whereas *pis1* is 36-fold more sensitive (Garbers *et al.* 1996; Fujito & Syono 1997). Each mutant also has a contrasting response towards different classes of auxin transport inhibitors. For example, *rcn1* roots curl in response to NPA but not TIBA, whereas *pis1* roots curl in the presence of either inhibitor, but not the morphactin, HFCA (Garbers *et al.* 1996; Fujito & Syono 1997). The contrasting responses of each mutant provide genetic evidence to support the observed biochemical and morphological differences which distinguish these classes of inhibitor (Lomax *et al.* 1995).

The *RCN1* gene product has been cloned and exhibits similarity to the regulatory subunit A of the protein phosphatase 2A (PP2A) enzyme (Garbers *et al.* 1996). This similarity has been demonstrated functionally by the *RCN1* sequences' ability to rescue the yeast PP2A mutant, *tpd3-1*. Hence the *RCN1* gene product may regulate efflux carrier activity by altering its degree of phosphorylation (see figure 4).

The *PIS1* gene may also act as a negative regulator of both the influx and efflux carriers (figure 4). This suggestion is based on the observation by Fujito & Syono (1997) that, in addition to an altered response towards selected auxin transport inhibitors, *pis1* root elongation exhibits an enhanced sensitivity towards the synthetic auxin, 2,4-D. 2,4-D is a known substrate of the influx, rather than the efflux, carrier (Delbarre *et al.* 1996). Alternately, *PIS1* activity may be influenced by the intracellular accumulation of auxins such as 2,4-D. Neither model is mutually exclusive because a plant cell would be expected to regulate its auxin concentration by coordinating the activities of both IAA influx and efflux carriers. In support of such a model, Delbarre *et al.* (1996) have reported that NPA can influence efflux and influx carrier activities within tobacco suspension cells.

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES