Plant hormones: **Ins and outs of auxin transport**

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Regulated transport has long been known to play a key part in action of the plant hormone auxin. Now, at last, a family of auxin efflux carriers has been identified, and the characterisation of one family member has provided strong evidence in support of models that have been proposed to explain gravitropic curvature in roots.

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The mechanism by which the hormone auxin regulates plant growth and development is a particularly exciting area of research at present, with rapid progress being made on several fronts. The latest advance is in the field of auxin transport, with the recent identification of a family of auxin efflux carriers [1–4]. The regulated, directional transport of auxin has been studied for many years (reviewed in [5]). Indeed, it was the 'basipetal' transport of auxin in stem segments — transport in the direction from the apex of the plant towards the roots — that allowed the initial purification of the most common natural auxin, indole-3-acetic acid (IAA). The use of specific inhibitors has established the importance of polar auxin transport in the control of plant growth and development. Detailed biochemical and physiological studies of auxin movements have led to a series of models for the roles of auxin transport, but these have remained controversial as, without a handle on the key cellular molecules that mediate the transport, they have been difficult to test definitively. The identification of a key component of the auxin efflux carrier will therefore have a major impact in moving the field forward.

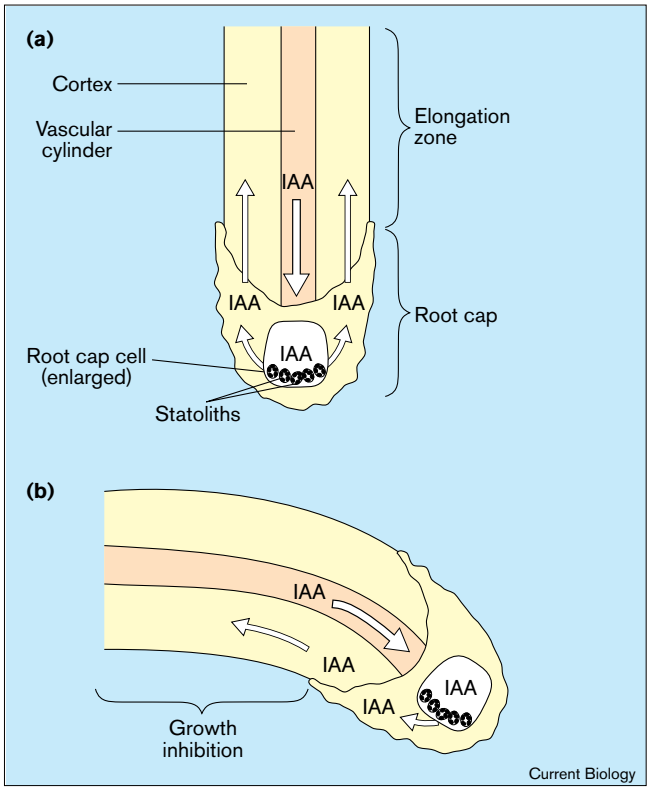
Polar auxin transport

In the early experiments that led to the first purification of auxin, auxin activity was found to accumulate in agar blocks that were placed on the basal surface of excised stem segments. If, in contrast, the block was placed on the apical end of an excised stem segment, then no such accumulation of auxin activity was observed. A modification of this experiment has become a mainstay of auxin transport research. Radio-labelled auxins are applied to the apical surface of stem segments and their arrival at the basal surface is monitored. This assay allowed the identification of compounds that specifically inhibit polar auxin transport, providing tools for the analysis of the roles of auxin transport in the plant.

Treatment of plants with auxin transport inhibitors has a wide range of effects [5]. Auxin transport inhibitors disrupt axis formation, vascular differentiation, apical dominance, organogenesis and tropic growth. The role of auxin transport in tropic growth is particularly noteworthy, as it has been suggested that tropisms — growth in a direction defined by some environmental cue, such as the direction of sunlight — are mediated by changes in auxin transport activity, although it is likely that changes in auxin sensitivity also play a role.

A good example of this is the direction of root growth, defined by the vector representing the force of gravity,

Figure 1



A model for the control of root elongation and growth in a direction defined by the gravity vector. **(a)** Auxin (IAA) is transported down to the root tip from the shoot in the vascular cylinder. Here it is redistributed to the root cortex and epidermis, and transported back up the root to the elongation zone, where it regulates the rate of cell elongation. **(b)** If the root is oriented orthogonal to the gravity vector, then the direction of the gravity vector can be detected by the sedimentation of starch grains (statoliths) in cells in the root cap. This, in turn, could lead to the asymmetric redistribution of auxin to the lower side of the root, where elongation is inhibited and the root consequently bends down in the direction of the gravity vector. It is likely that changes in auxin sensitivity are also involved in this process.

which is thought to be regulated by the redistribution of auxin transported down from the shoot by polar transport in the stele. At the root tip, the auxin is transported sideways into the epidermis and back up the root to the elongation zone, where it regulates the rate of cell elongation (Figure 1a). The gravity vector is sensed in the root cap, probably by the sedimentation of starch grains — commonly known as statoliths — and this influences auxin transport away from the root tip so that, if the long axis of the root is not parallel to the gravitational vector, auxin is asymmetrically redistributed toward the lower side of the root. The accumulation of auxin on the lower side inhibits elongation and results in the root bending downwards (Figure 1b).

The chemiosmotic hypothesis

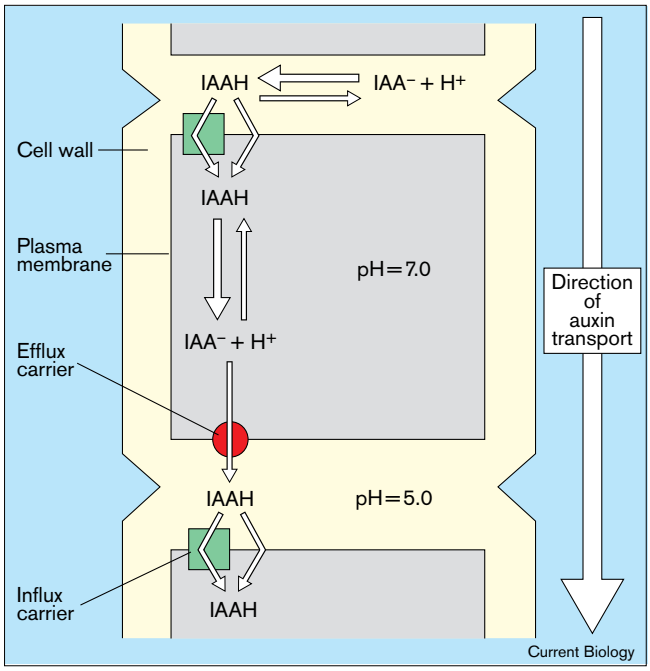
In addition to providing tools to study the physiological roles of auxin transport, transport inhibitors have been invaluable for biochemical investigations of auxin movement into and out of cells in culture. These studies have led a 'chemiosmotic' model of polar auxin transport (Figure 2) [5]. According to this model, auxin enters the cell either directly in its protonated form or through the action of a saturable auxin uptake carrier. On exposure to the cytoplasmic pH, auxin undergoes deprotonation, and in its consequently charged state it is effectively trapped inside the cell. Auxin can leave the cell only through the activity of an auxin efflux carrier, which is proposed to be localised specifically to the basal plasma membrane.

It is the basal localisation of the efflux carrier that is thought to drive polar transport. Evidence for this basal localisation has come from studying the site of action of auxin transport inhibitors of the so-called phytotropin class, such as 1-N-naphthylphthalamic acid (NPA). Phytotropins have been shown to block auxin efflux in a non-competitive manner, suggesting that they act at a separate site to that mediating the efflux catalytic activity. Indeed, the NPA binding site may be on a separate polypeptide, which may regulate the activity of the efflux carrier. Monoclonal antibodies that prevent NPA binding to pea microsomal membrane proteins were found to bind specifically to the basal plasma membrane of cells in the pea stem [6]. Until now, this was the only direct evidence for the basal localisation of the auxin efflux carrier, and unfortunately the antibodies were lost, preventing further analysis.

An auxin efflux carrier family

As with so many areas of plant biology, the study of auxin transport is now benefiting from genetic approaches in *Arabidopsis*. The isolation and phenotypic characterisation of auxin transport mutants is providing important new evidence, gratifyingly largely supporting the model described above, and the isolation of the mutationally defined genes is providing a molecular understanding of their action.

Figure 2



A model for polar auxin (IAA) transport involving basally localised auxin efflux carriers.

Support for the existence of a basally localised efflux carrier has come from the study of the *pin1* mutant of *Arabidopsis*. Loss of *PIN1* function results in morphological phenotypes that can be phenocopied by growth on media supplemented with auxin transport inhibitors. Consistent with a defect in auxin transport, *pin1* mutant plants show reduced polar auxin transport in the stem. The *PIN1* gene has recently been cloned by transposon tagging and is predicted to encode a transmembrane protein with some similarity to bacterial transporters including the arsB arsenic efflux carrier of *Escherichia coli* [3].

The PIN1 protein is localised to the basal membranes of cell files in the stem, as predicted for an auxin efflux carrier. *PIN1* is a member of a multigene family, and a particularly exciting recent development is the functional characterisation of a second family member, variously named *EIR1*, *PIN2*, *WAV6-52* or *AGR1* (referred to below as *EIR1*) [1,2,4]. Whilst *PIN1* acts in stem tissues, this second gene acts in the root tip, and evidence that it encodes an auxin efflux carrier comes from observations that heterologous expression of the gene in *Saccharomyces cerevisiae* confers resistance to toxic fluorinated indolic compounds and promotes efflux of radio-labelled auxin [1,4].

The *EIR1* gene was originally defined mutationally by loss-of-function alleles that confer a variety of phenotypes suggestive of a defect in auxin redistribution at the root

tip. These include an absence of root gravitropism and altered patterns of expression from an auxin-inducible promoter in the root tip. Immunolocalisation with antibodies against the EIR1 protein revealed a striking pattern of protein accumulation [2]. The protein was found to be limited to the plasma membranes of root tip cortical and epidermal cell files, starting three to five cells back from the centre of the root apical meristem. In cortical cells, the protein is localised at the membrane in contact with the epidermal cell file and in the membrane farthest away from the root tip. In the epidermal cells, it is localised only in the membrane farthest away from the root tip. This pattern of protein expression is entirely consistent with the model in which the localisation of the efflux carrier results in the redistribution of auxin arriving in the stele outward and back up the root in the epidermis and cortex (Figure 1).

Auxin transport and ethylene

The available data on *EIR1* fit well with existing models for auxin transport and its role in root gravitropism. The phenotype of loss-of-function *eir1* mutants has yielded some surprises, however. The gene is called *EIR1* because loss-of-function mutants have ethylene-insensitive roots: the elongation of their roots is resistant to the usual inhibitory effects of the plant hormone ethylene. This phenotype is epistatic to mutations in the *CTR1* gene of *Arabidopsis* that result in a constitutive response to ethylene and hence short roots (the CTR1 protein is thought to act as a negative regulator of ethylene signalling, downstream of ethylene perception).

Why an inability to redistribute auxin might suppress ethylene-mediated inhibition of cell elongation so late in the ethylene signalling pathway is unclear. Perhaps the inhibition of cell elongation by ethylene is mediated through auxin, and without EIR1 there is insufficient auxin in the elongation zone for ethylene to act. It is certainly true that many *Arabidopsis* mutants whose roots are resistant to the inhibitory effects of auxin are cross-resistant to ethylene, consistent with the hypothesis that ethylene acts through auxin. Of particular relevance here are mutants in the *AUX1* gene [7,8]. There is mounting evidence that *AUX1* encodes an auxin influx carrier required for auxin redistribution in root tips. Loss-of-function *aux1* mutations result in agravitropic roots that are also ethylene-resistant.

Families of auxin transporters

Reverse genetic approaches are currently being used to investigate the effects of mutations in other members of the auxin efflux gene family, with the aim of defining their specific roles in growth and development. The *AUX1* gene is similarly a member of a gene family in *Arabidopsis* [8], and other members of this family presumably have a similar range of roles in auxin transport.

The new results reviewed above illustrate again the power of the genetic approach. The continued identification of new auxin transport mutants is providing the opportunity for understanding its basis at a detailed molecular level. Of particular note are mutations in three *Arabidopsis* genes: *TIR3*, which result in reduced NPA binding [9]; *RCN1*, which cause resistance to some of the effects of NPA [10]; and *MP*, which disrupt canalisation of auxin transport [11]. These mutants, and others, are helping to solve another of the many intriguing biological questions that were first highlighted by Darwin [12] in the last century.

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